PAULO FILIPE MACHADO LOUREIRO

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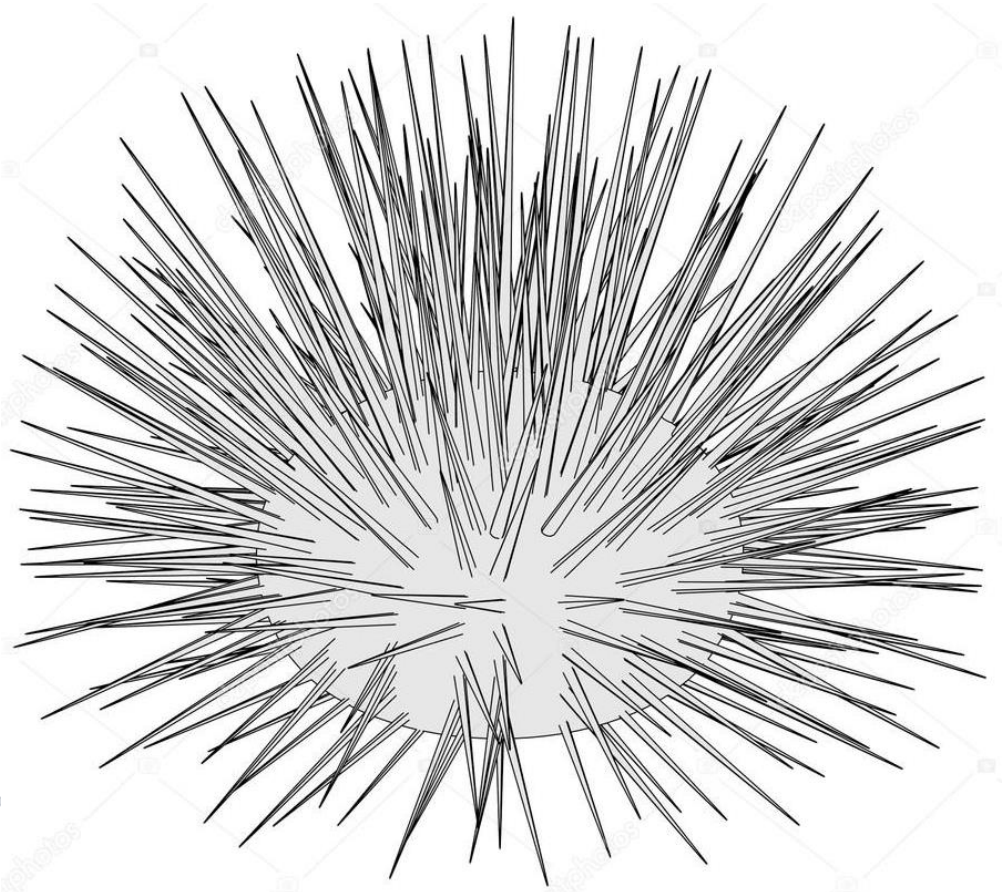

UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS E TECNOLOGIA

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Mestrado em Aquacultura e Pescas

Especialidade em Aquacultura

Trabalho efetuado sob a orientação de:

Supervisor: Pedro Marques Pousão-Ferreira

IPMA – Instituto Português do Mar e da Atmosfera

EPPO – Estação Piloto de Piscicultura de Olhão

Co-supervisor: Sofia Alexandra Dias Engrola

CCMAR – Centro de Ciências do Mar

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Declaração de autoria de trabalho:

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

(Paulo Filipe Machado Loureiro)

Vista aérea da Estação Piloto de Piscicultura de Olhão (EPPO) do IPMA onde foi realizado este ensaio.

Este trabalho foi financiado e realizado na Estação Piloto de Piscicultura de Olhão (EPPO) do IPMA utilizando ouriços-do-mar nascidos nesta Estação e as infraestruturas e meios disponíveis para o cultivo, amostragens biológicas e análises laboratoriais.

Acknowledgements

Queria agradecer desde já todas as pessoas que, de perto ou de longe, participaram e ajudaram na realização desta Tese de Mestrado, e especialmente:

- Ao Doutor Pedro Pousão-Ferreira, por me ter dado a oportunidade de realizar este trabalho nas suas instalações, no âmbito do projeto OURIÇAQUA (Fundo Azul), por ter confiando em mim quando me atribuiu este ensaio, e por ter sempre estado disponível para qualquer dúvida.

- À Doutora Sofia Engrola, por ter aceitado ser a minha cossupervisora, por todo o tempo dedicado a corrigir e a ajudar-me a tornar este trabalho o mais completo possível, mas também pela sua disponibilidade e simpatia.

- Ao João Araújo, sem quem este trabalho não teria sido realizado, pois acompanhou-me e ajudou-me ao longo da totalidade do ensaio. Queria agradecer-lhe por ter tornado as amostragens mais rápidas e divertidas, graças ao seu apoio, à sua boa disposição sem fim, e ao seu sentido do humor. Obrigado João por todo o tempo que investiste na minha aprendizagem, a responder às minhas mensagens e pelas inúmeras correções que fizeste e os conselhos que deste para a boa realização deste trabalho.

- À minha colega Laura Calvinho, pelo seu apoio incondicional, por me ter ajudado nas diferentes amostragens, e por me ter incentivado a levar a Tese de Mestrado até ao fim. A sua companhia foi fundamental, pois permitiu-me ultrapassar momentos mais complicados durante a dissertação deste trabalho, onde o stress e a ansiedade me invadiram.

- Aos meus pais e ao meu irmão, por me terem acompanhado ao longo da minha escolaridade, por terem acreditado em mim e apoiado nas minhas decisões sem qualquer interrogação. Queria agradecer ao meu pai, pois foi ele que partilhou comigo o seu amor pelo Mar, e sem quem nunca teria desenvolvido esta paixão pela biologia marinha e a aquacultura em geral. Queria igualmente agradecer à minha mãe, que, em 2015, me deu a excelente ideia de ir estudar para a Universidade do Algarve, mesmo que isso nos tenha afastado de 1500 km durante 5 anos. Mas como se diz em francês, “longe da vista, perto do coração”. Por último, queria agradecer o meu irmão por ter feito o seu papel de “irmão mais velho”, pois ajudou-me a crescer e a ganhar maturidade, fundamentais na minha

vida de universitário. Quero que saibam que tenho tanto orgulho em cada um deles, como eles de mim.

- À Gina, à Joana, ao senhor António, e sobretudo à dona Natália, por se terem tornado na minha segunda família e sempre me terem tratado como tal.

- Em último lugar, aos outros membros da EPPO, pela simpatia, pelo acolhimento, pela disponibilidade, e por estarem sempre prontos a ajudar. Pessoas simples, sinceras, com um grande coração. Muito obrigado Ana, Dra. Florbela, Marisa, Beta, Lurdes, Tetyana, Isa, Ana Catarina, Papitxi, e todos os outros com quem partilhei o meu dia a dia durante 6 meses!

Abstract

The purple sea urchin, *Paracentrotus lividus*, like other species of sea urchin, is of great economic interest, as its gonads are considered a delicacy. In certain countries, such as Japan, the sea urchin gonads, also known as "*uni*", are fully part of the local culture and gastronomy. To respond to the increase in demand, the last decades have been marked by an increase in the capture of wild sea urchins, but, as demand continued to increase and the capture of wild sea urchins was no longer ecologically sustainable, interest in echinoculture emerged. Various studies have shown positive results on somatic and gonadal growth when feed availability is high, nonetheless, the main obstacles encountered in the production of sea urchins are related to feed: difficulty in finding the balance between cost and quality of feed, limited growth due to inadequate diets and poorly optimized and efficient feeding regimes. Thus, it is essential to provide a high-quality feed since the main goal is to maximize production and obtain a final product of high commercial value. The aim of the present study was to determine the effects of two inert diets on somatic and gonadal growth of *P. lividus*, and to investigate the effects of sea urchin density on their feeding regime and somatic growth. For that, three trials were carried out, a 20-weeks nutritional trial under farm-conditions, with 450 sea urchins, and a 10-weeks nutritional trial under laboratory-conditions, with 180 sea urchins to determine the effect of two inert diets, containing 20% *Ulva* spp., on somatic and gonadal growth of juveniles *P. lividus*, and a 10-weeks density trial under lab-conditions, with 360 sea urchins, to investigate the effects of rearing density on growth and nutritional performances. At the end of the trials, *P. lividus* juveniles fed with inert diets showed higher total wet weight gain (TWWG), higher total specific growth rate (TSGR) and higher gonadosomatic index (GSI) than those fed with fresh *Ulva* spp. Sea urchins fed with inert diets showed 6 to 7 times lower feed conversion ratios (FCR) and 3 to 4 times higher protein efficiency ratios (PER) than sea urchins fed with fresh *Ulva* spp. The rearing density (268 ind.m^{-2}) had no impact on growth and nutritional performance of the sea urchins. This study has shown that inert diets containing 20% *Ulva* spp. are adequate for an echinoculture, under farm or lab-conditions. The diets were able to enhance somatic and gonadal growth, nutritional performance and with none detrimental impact in sea urchins' health.

KEYWORDS: Purple sea urchin (*Paracentrotus lividus*), Inert diet, Echinoculture, Aquaculture, Nutrition

Resumo

O ouriço-do-mar roxo, *Paracentrotus lividus*, assim como outras espécies de ouriço-do-mar, é de grande interesse econômico, pois as suas gónadas são consideradas uma iguaria. Em alguns países, como o Japão, as gónadas de ouriço-do-mar, também conhecidas como "*uni*", fazem parte da cultura e da gastronomia local, e são encontradas no comércio sobre várias formas: frescas, congeladas, desidratadas, salgadas ou cozidas. Na Europa, a França, a Espanha e a Itália são os maiores consumidores de *P. lividus*. Noutros países como Portugal, onde o consumo das suas gónadas não é tão comum, os ouriços-do-mar são exportados para suplementar o mercado dos países vizinhos, atingindo valores de cerca de 20€ por quilo, quando vendidos frescos, podendo os valores aumentarem consideravelmente quando processados. Para responder ao aumento da procura, as últimas décadas foram marcadas pelo aumento da captura de ouriços-do-mar selvagens, o que levou à sobre-exploração do recurso, e ao colapso das populações selvagens em várias áreas. Contudo, à medida que a procura continuou a aumentar e a captura de ouriços-do-mar selvagens deixou de ser ecologicamente sustentável, surgiu o interesse pelo cultivo de ouriços-do-mar. Na Europa, o ouriço-do-mar roxo, *Paracentrotus lividus*, é o principal candidato para o desenvolvimento do cultivo de ouriços-do-mar e faz parte de uma lista de espécies-alvo para a diversificação da aquacultura. Vários estudos têm mostrado resultados positivos no crescimento somático e gonadal quando a disponibilidade de alimentos é elevada, no entanto, os principais obstáculos encontrados na produção de ouriços-do-mar estão relacionados com a sua alimentação: dificuldade em encontrar o equilíbrio entre custo e qualidade da alimentação, crescimento limitado devido a dietas inadequadas e regimes alimentares pouco otimizados e eficientes. Assim, é essencial fornecer uma ração de alta qualidade pois o objetivo principal é maximizar a produção e obter um produto final de alto valor comercial. O objetivo do presente estudo foi determinar os efeitos de duas novas dietas inertes no crescimento somático e gonadal do ouriço-do-mar roxo, *Paracentrotus lividus*, em condições de campo e de laboratório, e, em paralelo, testar um novo meio de cultivo, inicialmente previsto para o cultivo de ostras, e investigar os efeitos da densidade de cultivo do ouriço-do-mar no seu regime alimentar e crescimento somático. Para tal, foram realizados três ensaios distintos, em condições de campo e em condições de laboratório. Em primeiro lugar, procedeu-se a um

ensaio nutricional de 20 semanas em condições de campo, com um total de 450 ouriços do mar. Os ouriços-do-mar foram separados em grupos de 50 indivíduos, onde se testaram três dietas diferentes, em triplicado. Assim, os ouriços-do-mar foram alimentados com uma das duas dietas inertes, contendo 20% de *Ulva* spp., realizadas pela empresa SPAROS, Lda., ou com uma dieta à base de *Ulva* spp. fresca e avaliou-se mensalmente o efeito destas dietas no crescimento somático (peso e diâmetro do teste) e gonadal (índice gonadossomático), mas igualmente no estado de maturação das gónadas. Em simultâneo, procedeu-se a um ensaio nutricional de 10 semanas em condições de laboratório, com 180 ouriços do mar, para determinar o efeito das duas dietas inertes no crescimento somático e gonadal de juvenis de *P. lividus* em comparação com a *Ulva* spp. fresca. O crescimento somático (peso) foi analisado a meio e no final do ensaio, e as performances nutricionais (taxa de conversão alimentar e taxa de eficiência proteica) foram analisadas no final da experiência. Para terminar, procedeu-se a um ensaio de densidade de 10 semanas em condições de laboratório, com 360 ouriços do mar, para investigar os efeitos da densidade de cultivo no crescimento e desempenho nutricional. Tal como no segundo ensaio, o crescimento somático (peso) foi determinado um mês após o seu início, e no final da experiência, juntamente com as performances nutricionais (taxa de conversão alimentar e taxa de eficiência proteica). No final dos ensaios, os juvenis de *P. lividus* alimentados com dietas inertes apresentaram maior ganho de peso húmido total (TWWG), maior taxa de crescimento específico total (TSGR) e maior índice gonadossomático (GSI) do que aqueles alimentados com *Ulva* spp. fresca, apoiando assim o facto de que as dietas formuladas favorecem o crescimento somático e gonadal dos ouriços-do-mar. O estudo histológico do estado de maturação das gónadas dos ouriços-do-mar não relevou diferenças estatisticamente significativas entre os tratamentos, mostrando assim que as dietas inertes suplementadas com 20% de *Ulva* spp. não influenciam a velocidade de maturação das gónadas, ou seja, não impacta o seu ciclo reprodutivo. Os ouriços-do-mar alimentados com dietas inertes apresentaram taxas de conversão alimentar entre 6 e 7 vezes mais baixas (FCR) e taxas de eficiência proteica entre 3 e 4 vezes mais altas (PER) do que os ouriços-do-mar alimentados com *Ulva* spp. fresca, o que é fundamental e determinante na viabilidade duma aquacultura. Para além disso, não houve qualquer diferença estatisticamente significativa na mortalidade dos ouriços-do-mar dos diferentes tratamentos. A densidade de cultivo não teve qualquer impacto na mortalidade, no crescimento e no desempenho nutricional dos ouriços-do-mar. Ambas as dietas inertes testadas neste trabalho foram bem aceites pelos ouriços-do-mar devido ao aumento da

palatibilidade característico da *Ulva* spp. suplementada nas rações. Dado os resultados obtidos ao longo dos diferentes ensaios, este estudo mostrou que dietas inertes suplementadas com 20% de *Ulva* spp., são adequadas para o cultivo de ouriços-do-mar, em condições de campo ou em condições de laboratório, melhorando o crescimento somático e gonadal, desempenho nutricional e sem qualquer impacto prejudicial na saúde dos ouriços-do-mar. Como os ouriços-do-mar se destinam para o consumo humano, estudos futuros deverão avaliar a composição de aminoácidos das gónadas, pois certos aminoácidos influenciam o sabor das gónadas. Em paralelo, também seria de grande interesse quantificar a perda de proteína da ração na água ao longo do tempo, e avaliar a capacidade de reprodução e a qualidade dos gâmetas dos ouriços-do-mar roxo, *Paracentrotus lividus*, alimentados com estas duas dietas inertes, para determinar se são adequadas à reprodução dos ouriços-do-mar e à obtenção de larvas.

PALAVRAS-CHAVE: Ouriço-do-mar roxo (*Paracentrotus lividus*), Dieta inerte, Cultivo de ouriços-do-mar, Aquacultura, Nutrição

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LIST OF UNITS AND ABBREVIATIONS

BW	Body weight
CTRL	Control
D1	Inert Diet 1
D2	Inert Diet 2
DE	Digestible energy
Dens1	Density 1
Dens2	Density 2
Dens3	Density 3
DM	Dry Matter
EPPO	Estação Piloto de Piscicultura de Olhão
FCR	Feed Conversion Ratio
GSI	Gonadosomatic Index
IPMA	Instituto Português do Mar e da Atmosfera
PER	Protein Efficiency Ratio
SGR	Specific Growth Rate
SU	Sea Urchin
TSGR	Total Specific Growth Rate
TWWG	Total Wet Weight Gain
WWG	Wet Weight Gain

LIST OF TABLES AND FIGURES

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1. INTRODUCTION

1.1 State of Aquaculture

Due to the demographic growth observed over the last decades, the consumption of fish in the world showed a considerable increase, of about 122% between 1980 and 2018, reaching *per capita* values of about 20.5 kg in 2018. In addition to being one of the healthiest foods, fish and fish products also have a reduced environmental impact compared to other food sources. In fact, fish brings essential nutritional contributions such as certain fatty acids, iodine, vitamin D, iron, calcium, zinc, and other minerals, and helps fight hunger and malnutrition in the world. Recently obtained data showed that, in 2017, 3.3 billion people satisfied 17% of their intake of animal protein thanks to fish consumption (FAO, 2020).

Fisheries data provided by FAO (2020) have shown some stability in recent decades as wild fish stocks are limited. For this reason, the last 30 years were mainly marked by a large increase in aquaculture, of approximately 530%. Without the products from aquaculture, it would not have been possible to respond to the increase in fish demand.

In addition to being an important source of food, aquaculture represents a great economic source. In 2018, world aquaculture produced 114.5 million tons of fish, for a commercial value of over USD 263 billion (FAO, 2020).

Currently, market and consumer demand, climate change and reduced availability of a species in the wild are the main reasons of the necessity for diversification of the species reared in aquaculture (FAO, 2016).

One of the species with great potential for diversification in aquaculture is the sea urchin, whose gonads are considered as a luxury food in several countries. In the last years, studies on this species, in the context of developing techniques for its rearing in aquaculture, have been multiplying (Pinna, 2014).

1.2 Description of the species

The edible sea urchin, *Paracentrotus lividus* (Lamarck, 1816) is a Regular Equinoid that belongs to the Parechinidae Family and the Camarodonta Order.

Sea urchins can be easily identified due to its pentameric radial body covered with spines, which can vary in size, diameter and color depending on the species.

Table 1.1. Classification of *Paracentrotus lividus* (Lamarck, 1816).

Taxon	Name	Author
Phylum	Echinodermata	Bruguière, 1790
Class	Echinoidea	Leske, 1778
Subclass	Euechinoidea	Bronn, 1860
Infraclass	Carinacea	Kroh & Smith, 2010
Superorder	Echinacea	Claus, 1876
Order	Camarodonta	Jackson, 1912
Infraorder	Echinidea	Kroh & Smith, 2010
Family	Parechinidae	Mortensen, 1903
Genus	*Paracentrotus*	Mortensen, 1903
Species	*Paracentrotus lividus*	Lamarck, 1816

In the case of the edible sea urchin, *P. lividus*, the spines are typically purple, green, red, black, or brown (Fig. 1.1) and constitute the sea urchin's main defense against predators, but they also serve to maximize adhesion to the substrate and participate in its locomotion (Guidetti & Mori, 2005). In addition to the spines, its pseudo-circular shell, commonly called test, also has other appendages such as tube feet and pedicellariae, with specific functions. Tube feet primarily serve for locomotion and fixation to the substrate. As they are highly flexible and can stretch and shrink depending on the situation, it allows the sea urchin to adhere the substrate thanks to their sucker-like tips. The main feature that distinguishes pedicellariae from tube feet is the shape of the tip. In fact, pedicellariae are small appendages whose tip is wrench-shaped and whose jaws are movable, which allows the sea urchin to remove or place debris, like macroalgae, pebbles, and shells, on its test (Flammang, 1996). The test is composed of 10 ambulacral plates, where the podium are located, and 10 interambularal plates, where the spines are located (Fig. 1.2), alternating between them (Carboni, 2013).

Fig. 1.1. Purple sea urchin, *Paracentrotus lividus* (Lamarck, 1816).

Another common point among all Regular Echinoids is the presence of the “Aristotle's lantern” in the mouth region. Aristotle's lantern is a set of 5 mobile plates arranged in a circle, each one hiding a tooth. This structure allows the sea urchin to feed, but also to dig holes in the rocks to hide from predators and protect themselves from waves hydrodynamics (Barnes, 1987; Tortonese, 1965).

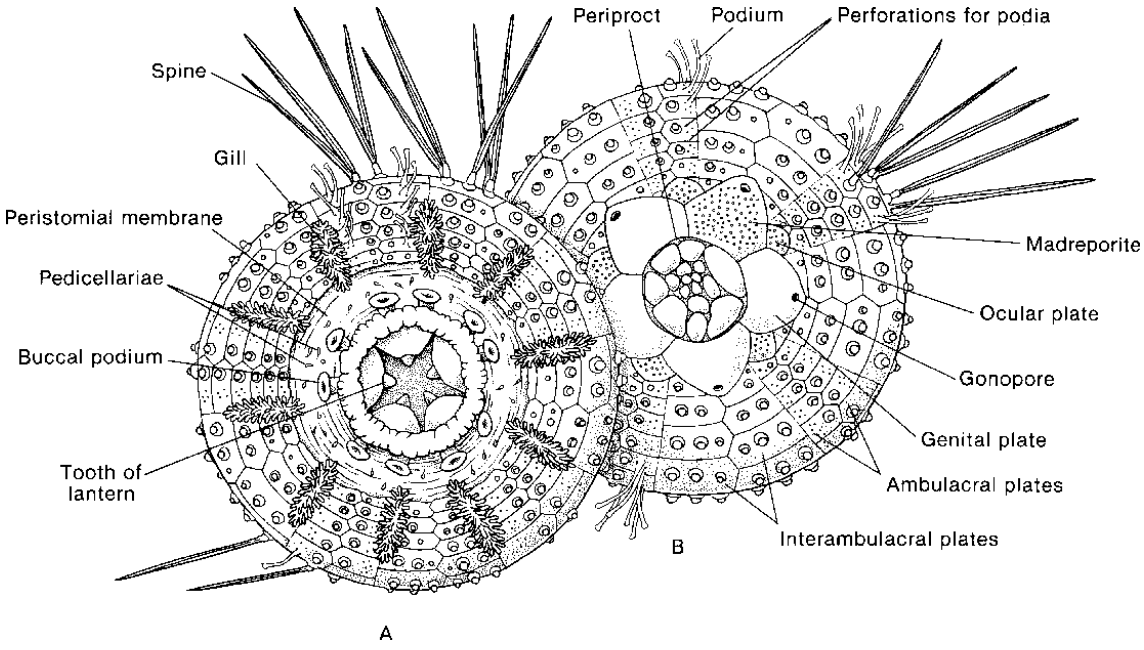

Fig. 1.2. External anatomy of a Regular Echinoid. A. Oral view. B. Aboral view. (from Grosjean & Jangoux, 2001)

The purple sea urchin, *P. lividus*, is a gonochoric and oviparous species, as fertilization takes place directly in the water column, where the gametes were previously released. They have 5 gonads, each directly linked to a gonopore, via a gonoduct (Fig. 1.3). When the gonads reach maturity, they usually vary between yellowish, orange, and reddish (Araújo *et al*., 2020; Carbonara *et al*., 2019).

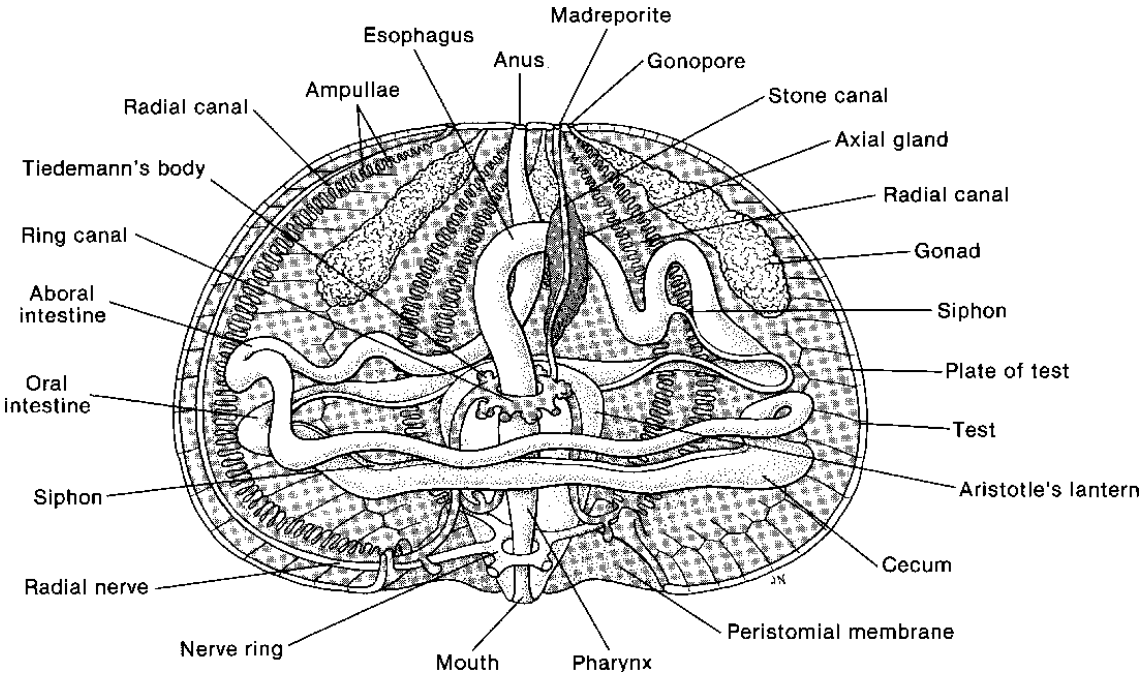

Fig. 1.3. Internal anatomy of a Regular Echinoid. Side View. (from Grosjean & Jangoux, 2001).

1.3 Biology and habitat of *Paracentrotus lividus*

The edible sea urchin, *Paracentrotus lividus*, is widely distributed along the coast of the Mediterranean Sea and the North-East Atlantic, where it is common to find it in rocky and shallow areas, from Scotland, to the south of Morocco (Cirino *et al*., 2017; Bertocci *et al*., 2014; Fernandez & Boudouresque, 2000; Ciriminna *et al*., 2020; Mendes *et al*., 2018, 2019; Araújo *et al*., 2020).

As it is an herbivorous and benthic species, it is usually found in the first 30 m of depth, close to macroalgal forests, where its food is abundant. Its distribution and density are closely related to physical and biological factors like the light and waves exposure, availability of food, predation, and larval survival (Guidetti *et al*., 2003).

Given its feeding behavior, the edible sea urchin is considered a major actor in the regulation of coastal macroalgal communities and local biodiversity (Farina *et al*., 2020). In fact, in areas where *P. lividus* populations have collapsed, there has been accelerated development of brown algae and notable changes in benthic and fish biodiversity. Conversely, in areas where the density of *P. lividus* was abnormally high, overgrazing occurred, which transformed these seabeds into desert areas (Gago *et al.*, 2003; Farina *et al*., 2020).

1.4 Economic and nutritional interest

The purple sea urchin, *P. lividus*, like other species of sea urchin, is of great economic interest, as its gonads are considered a delicacy (Cyrus *et al*., 2014; Carboni *et al*., 2012, Cirino *et al*., 2017; Ciriminna *et al*., 2020; Mendes *et al*., 2018, 2019, 2020; Araújo *et al*., 2020). In fact, the high economic value of the sea urchin is justified by the organoleptic characteristics much appreciated in Mediterranean, South American, and Asian countries (Cirino *et al*., 2017; Cyrus *et al*., 2014; Santos *et al*., 2020).

In certain countries, such as Japan, the sea urchin gonads, also known as "*uni*" (Fig. 1.4), are fully part of the local culture and gastronomy. With nearly 126 million inhabitants, Japan is the largest consumer of sea urchins in the world: it is estimated that 64% of the world's sea urchin production is consumed there (FAO, 2012). Meanwhile, Chile, with about 6,435 km of coastline, is the largest producer of sea urchins, with more than 55,000 tons per year (Carboni *et al*., 2012).

Fig. 1.4. Fresh sea urchin gonads.

In Europe, France, Spain, and Italy are the main consuming countries for sea urchin gonads, where their demand has increased considerably in recent decades (Bertocci *et al*., 2014; Santos *et al*., 2020; Mendes *et al*., 2018, 2019, 2020; Araújo *et al*., 2020). According to FAO data (2012), Spain is the country of the Eastern Atlantic with the highest landing of *P. lividus*, however, it is estimated that France has the largest market for sea urchins.

Even if not consumed locally, sea urchins are of economic interest as they can be exported to countries where they are consumed. Several countries export *P. lividus*, such as Croatia, Ireland, and Portugal (Bertocci *et al*., 2012; Boudoresque & Verlaque, 2007).

In Portugal, the consumption of sea urchins is rare and limited to some regions located on the coast, however, there is an export to neighboring countries, such as Spain and France, to supply their markets (Mendes *et al*., 2018, 2020).

The commercial value of sea urchins in Europe varies depending on several factors such as the species, the season, or the country (Carboni *et al*., 2012). Thus, each sea urchin can be sold at a price between 0.3 € and 3 €, or about 20€ per kilo (Hagen, 1996; Mendes *et al*., 2020; Carboni *et al*., 2012). Their gonads are usually consumed fresh; however, they are also commonly found frozen, dried, salted, or cooked. When processed, their gonads, considered as one of the most expensive seafood, can reach values in the order of 150€ per kilo (Carboni *et al*., 2012; Grosjean *et al*., 1998).

The other interesting aspect of sea urchin is that fat occupies an important place in human nutrition, as it is a fundamental component of cell membranes, hormones, and represents a considerable source of energy. As the human body cannot synthetize certain essential fatty acids (EFAs), such as some n-3 and n-6 PUFAs, they must be consumed directly in the diet. This lack of PUFAs can be fulfilled with seafood, like sea urchins, as seafood in general is known to be an important source of those polyunsaturated fatty acids (Taşbozan & Gökçe, 2017). In previously conducted studies, the fatty acids profile of *P. lividus* gonads was analyzed, and the results obtained showed that almost 45% of those fatty acids are polyunsaturated (De la Cruz-García *et al*., 2000; Mendes *et al.*, 2020). The consumption of those fatty acids is very important as a relationship was found between certain ω3 PUFA (eicosapentaenoic acid - 20: 5 ω3 & docosahexaenoic acid - 22: 6 ω3) and low mortality due to certain diseases, particularly cardiovascular ones (Simopoulos, 2002).

1.5 Fishing increase and overexploitation of wild populations

To respond to the increase in demand, the last decades have been marked by an increase in the capture of wild sea urchins (Carboni *et al*., 2012; Mendes *et al*., 2018, 2019, 2020; Araújo *et al*., 2020). Its global catch increased by about 2.5 times between 1982, with 48,000 tons, and 1995, with 120,000 tons (Carboni *et al*., 2012), thus reaching an over exploitation of the resource that negatively impacted local populations in various areas such as on the Ustica island (Western Mediterranean, Italy), northwestern Sardinia (Italy) and in the north of Portugal (Gianguzza *et al*.,2006; Pais *et al*., 2012; Bertocci *et al*., 2014). One of the main reasons that this species is overexploited is that, as sea urchins are caught before they are fully sexually mature, there is insufficient recruitment, which leads to an accelerated decline in stocks (Grosjean & Jangoux, 2001).

In addition, the impact of sea urchins overfishing has been exacerbated by habitat destruction, global warming, natural diseases, competition for resources with other species and poor fisheries management (Santos *et al*., 2020; Mendes *et al*., 2019).

1.6 Increasing interest in sea urchins

As demand continued to increase and the capture of wild sea urchins was no longer ecologically sustainable, interest in echinoculture emerged (Cyrus *et al*., 2014, Santos *et al*., 2020; Mendes *et al*., 2018, 2020; Araújo *et al*., 2020). In fact, echinoculture, in addition to responding to the demand increase, has helped to relieve pressure from fishing in wild populations (Ciriminna *et al*., 2020). In Europe, the sea urchin, *Paracentrotus lividus* is the target species in the development of echinoculture and is part of a list of target species for the diversification of aquaculture (Carboni *et al*., 2012; Santos *et al*., 2020). In 2016, the annual production of *Paracentrotus lividus* in aquaculture was 10 tons, while the capture of wild *P. lividus* was 10 times higher (Santos *et al*., 2020). This means that the culture of this species has a large margin of development.

In addition to sea urchins having a true economic value, there is also a scientific interest. In fact, certain studies aimed to control the reproductive cycle of sea urchins, in order to ensure available gametes throughout the year, for laboratory purposes such as ecotoxicology and developmental biology (Cirino *et al*., 2017).

For a successfully development of *P. lividus* culture, perfect knowledge of the species is essential (Santos *et al*., 2020). For this reason, scientific studies on sea urchins have been increasing rapidly, to develop effective cultivation methods, different feeding strategies, and new ways to maximize somatic and gonadal growth (Cyrus *et al*., 2014, Carboni *et al*., 2012; Ciriminna *et al.*, 2020; Santos *et al*., 2020).

1.7 Culture system and density

In trials realized previously, sea urchins were kept in plastic baskets suspended in tanks (Cyrus *et al*., 2014; Cirino *et al*., 2017). In comparison with these baskets, and because they are closed on both sides, the bags intended for the growth of oysters can withstand more dynamic environments, in addition to providing a greater area for sea urchins (Fig. 1.5). In one of the trials, it was shown that the increase in rearing density is directly related to the decrease in the survival rate in the sea urchin *Tripneustes gratilla* (Cyrus *et al*., 2014). For *P. lividus*, no information was found on the most suitable rearing density for a viable large-scale echinoculture.

Fig. 1.5. Oyster baskets tested for *Paracentrotus lividus* culture

1.8 Sea urchin feeding

In sea urchins, somatic and gonadal growth depends on water temperature, photoperiod, and nutritional factors such as availability, quantity, and quality of feed (Cirino *et al*., 2017; Santos *et al*., 2020). The results obtained in a previously performed trial showed a better somatic and gonadal growth of sea urchins at 22ºC (Santos *et al*., 2020). The same factors also influence the gametogenic cycle of this species, with earlier gametogenic

stages when the temperature is below 18ºC, and greater sexual maturation in individuals fed with formulated diets and maintained at 20-22ºC. (Cirino *et al*., 2017; Santos *et al*., 2020). In an outdoor aquaculture, without using water heating and cooling, the water temperature and the photoperiod vary according to the season, as in the natural habitat, that is, of the three main factors previously mentioned, the feeding is the only parameter that differs from the natural habitat.

Sea urchins are essentially herbivores, feeding mainly on macroalgae, however, some studies have shown that they are opportunistic feeders and can also feed on sponges and other sea urchins' corpses (Queiroz, 2020; Cyrus *et al*., 2014; Fernandez & Boudouresque, 2000; Ciriminna *et al*., 2020; Santos *et al*., 2020). The sea urchin, *Paracentrotus lividus*, feeds on, among other species, *Ulva* spp., *Laminaria ochroleuca*, *Fucus vesiculosus*, and *Sargassum muticum* (Cardoso *et al*., 2020).

Various studies have shown positive results on somatic and gonadal growth when food availability is high (Lawrence *et al*., 1992; Gago *et al*., 2001), nonetheless, the main obstacles encountered in the production of sea urchins are related to feed: difficulty in finding the balance between cost and quality of feed, limited growth due to inadequate diets and poorly optimized and efficient feeding regimes. Its commercial value can also be affected as certain diets can degrade the nutritional and organoleptic characteristics of the gonads (Cirino *et al*., 2017; Ciriminna *et al*., 2020; Santos *et al*., 2020).

The use of macrophytes to feed sea urchins in an echinoculture has several disadvantages (Ciriminna *et al*., 2020). First, the costs associated with transporting the seaweed and storing it are very high and, in a large-scale echinoculture, the amount of macroalga needed to feed sea urchins represent a very high investment because it requires large spaces to be stored (Ciriminna *et al*., 2020). In addition to the economic aspect, their availability and nutritional characteristics vary depending on the season and location as shown in some studies (Schlosser *et al*., 2005; Fernandez & Boudouresque, 2000; Ciriminna *et al*., 2020, Santos *et al*., 2020; Mendes *et al*., 2020). In the study released by Schlosser *et al*. (2005), the total protein of *Ulva lactuca* was 374 mg/g in the Autumn, and 340 mg/g in the Spring; this decrease was also observed in the lipids of *Ulva lactuca*, with a total of 25 mg/g in the fall and 20 mg/g in the latter.

The negative aspects in the use of macroalgae in the feeding of sea urchins in aquaculture led to an increase in scientific research focused on their feeding, whose work found a

common solution: the development of formulated diets (Cirino *et al*., 2017; Santos *et al*., 2020).

In fact, inert diets are easier to store, can be kept at room temperature and allow greater feed control because the nutritional characteristics of the feed are known and invariable throughout the year (Fernandez & Boudouresque, 2000). In parallel, several studies have shown greater somatic and gonadal growth in sea urchins when fed with inert diets (Cyrus *et al*., 2014; Schlosser *et al*., 2005). In the trial conducted by Schlosser *et al*. (2005), sea urchins fed with formulated diets showed a digestible energy intake (DE) higher than sea urchins fed with different algae (*Ulva lactuca* and *Gracilaria conferta*), resulting in greater gonadal growth and respective gonadosomatic index.

Rapid growth and good development of the gonads are not the only factors to be considered when defining the feed strategy in sea urchins. Several studies have shown that the use of high-quality feed is directly related to an increase in the intake rate. The ingestion rate, associated with the digestion rate, are determining factors in the viability of an echinoculture, as feeding represents more than half of the costs associated with this culture (Cyrus *et al*., 2014). Likewise, the quality of the feed is closely related to the biochemical composition of the gonads and reproductive capacity (Cirino *et al*., 2017; Fernandez & Boudouresque, 2000; Santos *et al.*, 2020; Schlosser *et al.*, 2005), which is fundamental in its use in the laboratory, and with organoleptic characteristics, which will determine its marketing and its price.

However, providing a high-quality feed is essential since the main goal is to maximize production and obtain a final product of high commercial value.

To increase the consumption rate of a feed, it is important to increase its palatability (Cyrus *et al*., 2014). Previous studies have shown that the incorporation of seaweed in an inert feed act as a feed attractant, thus increasing the feeding ingestion of sea urchins, without, however, changing the nutritional value of the feed (Cyrus *et al*., 2014).

Ulva spp. is one of the most used macrophytes in the sea urchin feeding trials. In the species *Tripneustes gratilla*, a significant preference was found for this macroalga when incorporated into the formulated diets, and the associated results showed a more pronounced feeding ingestion, greater acceptability of the feed and a higher protein intake (Cyrus *et al*., 2014). Due to the good results obtained in *T. gratilla*, and as no preference has been determined in *P. lividus* feeding behavior fed with several red and brown

macroalgae (Cardoso *et al*., 2020), *Ulva* spp. was chosen to integrate the formulated diet used in the present study.

1.9 Protein and energy

Energy and protein are the two main factors that contribute to somatic and gonadal development in sea urchins (Cyrus *et al*., 2014). In general, the inert feeds given to sea urchins are composed of between 20 and 40% of protein, and their digestibility is over 60% (Schlosser *et al*., 2005).

Studies previously released have found that sea urchins with increased protein consumption had an increased somatic and gonadal growth, however, when this dietary protein consumption was too high, sea urchins showed a lower feed ingestion, which may mean that dietary protein requirements were fulfilled (Fernandez & Boudouresque, 1998; Schlosser *et al*., 2005; Heflin *et al*., 2012; Heflin *et al*., 2016).

Carbohydrates are used by sea urchins as the main energy source (Marsh *et al*., 2013). The addition of appropriate levels of dietary carbohydrates in inert feed increase somatic growth, gonadal growth, and nutrient utilization. Contrastingly, inappropriate levels of dietary carbohydrates in diet will have a negative effect on sea urchins: insufficient levels of dietary carbohydrates will limit metabolic processes, while excessive levels of dietary carbohydrates will requisite additional energy to process the surplus, by storing or excreting the excess (Heflin *et al*., 2016).

The aim of the present study was to determine the effects of two inert diets on somatic and gonadal growth of *Paracentrotus lividus*, and to investigate the effects of sea urchin density on their feeding regime and somatic growth, under farming conditions.

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2. MATERIALS AND METHODS

2.1 Collection, acclimatization, and maintenance of sea urchins

The juveniles of *Paracentrotus lividus* used in the present trial originated from the broodstock housed at the IPMA facilities (EPPO, Olhão), and were, at the time of collection, between 18 and 19 months old. For better acclimatization, the sea urchin juveniles were kept for 16 months in a 300-l raceway tank, properly oxygenated and filtered sea water, at room temperature. To avoid nutritional differences between individuals, they were fed a diet exclusively composed of fresh *Ulva* spp. and corn (*Zea mays*). In farm-conditions fattening trial and in lab-conditions nutrition trial, a total of 645 individuals participated, with the same range of size [2.0-2.9] cm in diameter and weight > 8 g.

2.2 Preparation of experimental diets

The two inert diets tested in the trials were developed and manufactured by the company SPAROS, Lda. (Olhão, Portugal). Specially designed for equinoculture, they had a disc shape (Fig. 2.1), and incorporated *Ulva* spp. to enhance the acceptance of sea urchins (Table 2.1).

Fig. 2.1 Inert diet tested in the present trial

The main difference between the two inert diets was the texture after immersion in water: Diet 1 had wheat meal in its composition, which was replaced in Diet 2 by potato starch and two other ingredients with binding properties, sorbitol, and sodium alginate.

Table 2.1. Ingredients and proximal composition of formulated diets fed to *Paracentrotus lividus*

Ingredients and proximal composition, % of total dry matter	**Diet 1**	**Diet 2**
Fish gelatin	5.00	5.00
Macroalgae (*Ascophyllum nodosum*)	20.00	20.00
Macroalgae (*Ulva* spp. supplied by IPMA)	20.00	20.00
Wheat gluten	7.50	7.50
Corn gluten meal	17.00	17.00
Wheat meal	10.00	
Potato starch (gelatinized)		5.00
Sorbitol		2.50
Vitamin and mineral premix	2.00	2.00
Antioxidant	0.40	0.40
Monocalcium phosphate	3.00	3.00
Calcium carbonate	5.00	5.00
Binder (sodium alginate)		2.50
Beta-carotene 10%	0.50	0.50
Algae biomass (*Schizochytrium* 16%DHA)	9.60	9.60
Total	**100.00**	**100.00**
Protein (%)	32.62	31.22

Fat (%)	5.19	5.06
Moisture (%)	5.67	6.44
Gross Energy (kJ g^{-1})	15.75	15.93
Ash (%)	21.00	21.37

In this assay, the diet used in Control treatment was *Ulva* spp. As its availability and its biochemical parameters vary throughout the year the total amount of algae needed for the trials was collected before the experiment started, washed and frozen in plastic bags with 500 g of seaweed per bag (20 bags) (Appendix I). A sample of *Ulva* spp. was collected, lyophilized, and kept at a temperature of -80ºC, however, due to internal issues on nutritional analysis, the proximate composition of *Ulva* spp. used in the present work was the one found by Ferreira *et al.* (2021), whose *Ulva* spp. came from an integrated multi-trophic aquaculture at Ria de Aveiro (Portugal) (Table 2.2).

Table 2.2. Proximal composition of fresh *Ulva* spp. (% DM) (from Ferreira *et al*., 2021)

Proximal composition of *Ulva* spp.	
Dry matter (%)	87.16 ± 0.01
Crude protein (%)	22.13 ± 0.08
Crude fat (%)	1.63 ± 0.10
Ash (%)	30.34 ± 0.04
Gross energy (kJ g^{-1})	11.03 ± 0.28

2.3 Feed Stability

Before starting the trials and integrating the inert diets into the sea urchins' alimentation, their behavior in water at 19ºC was tested. In the first 2 days, the formulated diets maintained their firmness, however they became softer on the third day. Between the third and the fourth day, both inert diets showed traces of bacterial activity. To avoid any risk due to these bacteria, it was decided to renew the diet every 3 days.

To quantify a possible leaching of the formulated diets in the water, 15 disks of each inert diet were submerged in sea urchin rearing water. Samples (3 disks) were removed after

60 s, 3 h, 6 h, 24 h and 48 h, which were sent for analysis at the IPMA laboratories in Lisbon.

2.4 Experimental conditions

Both tests were carried out at the Pilot Fish Farming Station in Olhão (EPPO) (Olhão, Portugal). Temperature and dissolved oxygen concentration were measured daily, using the HANNA HI98196 multiparameter.

2.4.1 Farm-conditions trial: The fattening of *Paracentrotus lividus* juveniles

A first sorting was carried out to select 464 sea urchins of the same size class [2.0-2.9] cm in diameter and weight > 8 g. A random biometric sampling of 100 individuals was carried out (carapace diameter and total weight). Furthermore, 14 individuals, randomly chosen, were previously placed in cold water (-1ºC), and then sacrificed to determine the initial gonadosomatic index (GSI). To determine the gonadosomatic index, each individual was weighed before dissection (total weight) and their gonads after dissection.

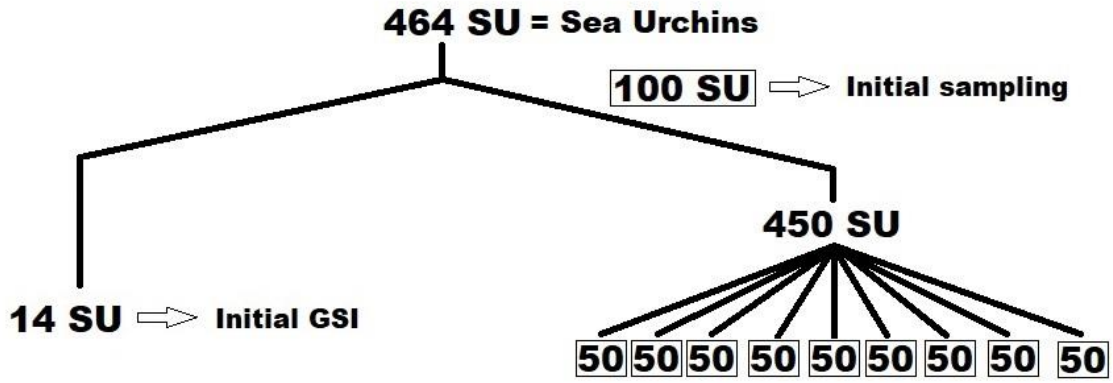

Fig. 2.2. Initial sampling of *Paracentrotus lividus* juveniles, farm-conditions trial (SU = Sea urchin).

The remaining 450 sea urchins were distributed to groups of 50 individuals in 9 oyster baskets (3 treatments in triplicate), with a biomass of 0.643 ± 0.034 kg. Then, the 9 baskets were distributed in 3 circular fiberglass tanks (volume of 3000 l) with one basket for each treatment per tank; were hung horizontally on a rope, on the first few cm of the water column. To reduce the luminous intensity, all tanks were covered with a shading net.

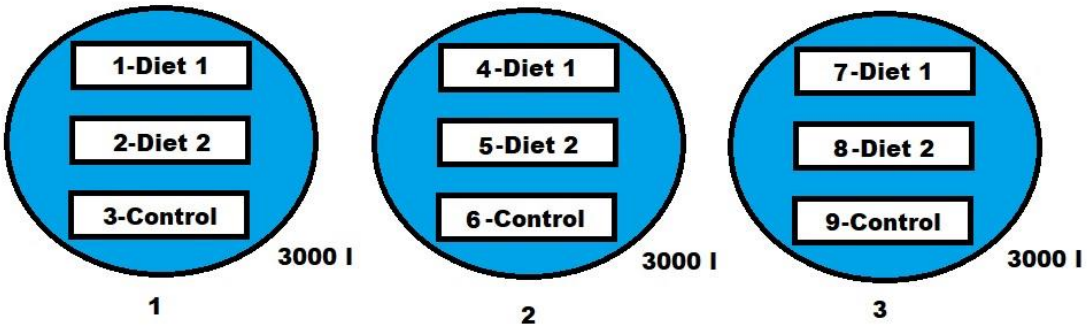

Fig. 2.3. Experimental design of farm-conditions trial.

Two phases took place before the start of the trial and the introduction of the different diets: an acclimatization phase during the first three weeks after being placed in the new tanks, where they were fed with *Ulva* spp. once a week, and a starving phase for about two weeks, aiming to cleanse the organism and increase appetite and acceptance of inert diets. Before the introduction of the different diets, a sampling was performed to verify that there were no differences between treatments (Table 2.1).

Table 2.3. Average test diameter (cm) and wet weight (g) of *Paracentrotus lividus* from the three treatment groups, before the introduction of the diets (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.). Letters a, b, c ($p<0.05$) represent a significant difference in the means of sea urchins fed the different diets.

	T=0		
	D1	D2	CTRL
Test diameter (cm)	3.48 ± 0.41^{a}	3.39 ± 0.39^{a}	3.50 ± 0.36^{a}
Wet weight (g)	13.66 ± 3.31^{a}	12.19 ± 3.41^{a}	13.78 ± 3.53^{a}

n=30. (mean ± SD). Same letter in the line stand for no significative differences ($p>0.05$).

The determination of the quantity of feed supplied was made considering the biomass of sea urchins in the baskets and the existing bibliography (Gibbs *et al*., 2015). The feeding regime consisted of 50 g of inert Diet 1 for sea urchins in baskets 1, 4 and 7, 50 g of inert Diet 2 for sea urchins in baskets 2, 5 and 8, and 55 g of *Ulva* spp. for sea urchins in baskets 3, 6 and 9, every 3 days; the amount of feed supplied was of the order of 2.5% $BW.d^{-1}$. On the third day after each feeding, the uneaten feed was removed, and new feed replaced.

The amount of feed provided in baskets 3, 6 and 9 (control) was increased to ensure that all sea urchins ate *ad libitum*.

Biometric sampling (total weight and carapace diameter) of 30 sea urchins per basket was performed once a month. Weighing was done with a KERN PRS / PRJ precision and analytical balance. To measure the diameter of the carapace, the sea urchins were photographed next to a ruler, and the pixel-centimeter conversion was performed using the ImageJ software (Appendix II). At the same time, and for each basket, 3 randomly chosen individuals were weighted, and then, anesthetized by submersion for five minutes in cold water (-1ºC), to proceed with their dissection. With a scalpel, a circular opening was made at the bottom of the sea urchin, and the feces were removed with a slight jet of distilled water (Appendix III). Thus, each individual's gonads were extracted and weighed to determine the gonadosomatic index.

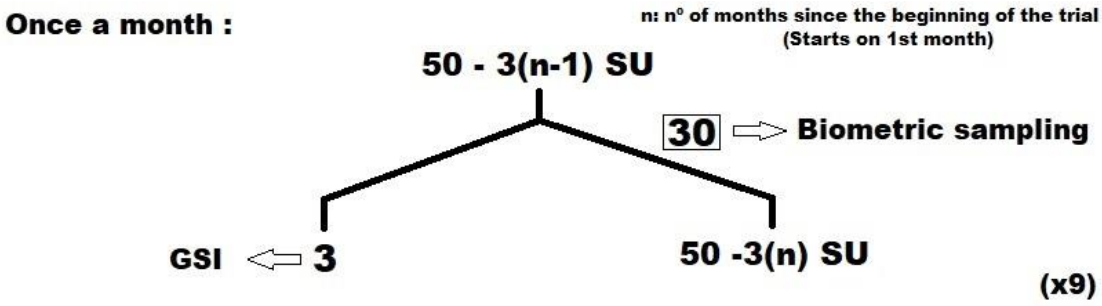

Fig. 2.4. Monthly sampling of *Paracentrotus lividus* juveniles, farm-conditions trial (SU = Sea urchin).

During the final sampling, 2 male sea urchins and 2 female sea urchins per basket were randomly selected for histological analysis of the gonads. As previously described, individuals were anesthetized by submersion for five minutes in cold water (-1ºC) to proceed with their dissection. One gonad was extracted per sea urchin, and a total of two gonads, from individuals of the same treatment and of the same sex, were placed in each properly labeled tissue embedding cassette. Tissue embedding cassettes were immediately placed in 10% formaldehyde for 48h for fixation and transferred to 70% ethanol for storage until paraffin histology.

This trial was performed for 5 months.

2.4.2 Laboratory conditions feeding trial

For this trial, 180 individuals of the same category were selected, with similar carapace diameter and total weight. Nine groups of 20 sea urchins were composed (3 treatments with triplicates), and no statistically significant differences were found in the initial biomass of the different treatment groups, with a mean initial biomass of 259.8 ± 3.28 g.

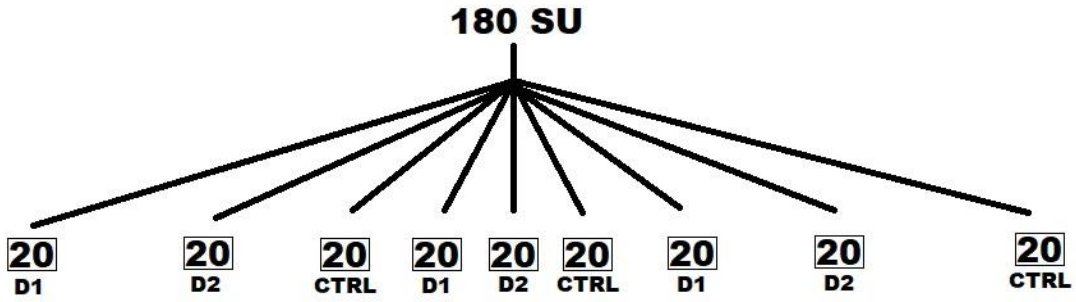

Fig. 2.5. Experimental design of laboratory-conditions nutrition trial (SU = Sea urchin).

At first, the sea urchins were placed in 20 l circular tanks for 14 days, without feed, to go through a phase of acclimatization and starvation, thus favoring the acceptance of inert diets. During this phase, after observing the behavior of sea urchins, it was concluded that the tanks used would not be the most suitable, as their feed would remain at the bottom, when they preferred to be on the sides of the tank, with, thus, an insufficient contact between the individuals and the feed. To reduce the vertical profile of the sea urchins' growing medium and increase their contact with the feed, rectangular tanks (38 l) with more horizontal space were used and the sea urchins were placed between 2 boxes of plastic, inside the respective tanks.

In this trial, to elaborate the feed plan, all known factors previously applied in the outdoor trial were considered, and the amount of feed provided was slightly decreased, to 1.96 ± 0.04% $BW.d^{-1}$. Every 3 days, the tanks were cleaned, uneaten feed was determined and removed, and new feed was accurately weighed and replenished.

All sea urchins, from each treatment, were weighed individually at the beginning and at the end of the trial. An intermediate weighing of the 20 sea urchins in each tank was carried out, one month after the beginning of the experiment, to follow the evolution of biomass.

This trial lasted 10 weeks.

2.4.3 Laboratory-conditions density trial

For this trial, 360 individuals of the same category were selected, with similar carapace diameter and weight, > 7 g. There were 3 groups of 20 sea urchins, 3 groups of 40 sea urchins and 3 groups of 60 sea urchins. The configuration of the tanks was the same used in the lab-conditions feeding trial: 38 l rectangular tanks, properly oxygenated and continuous filtered water arrival.

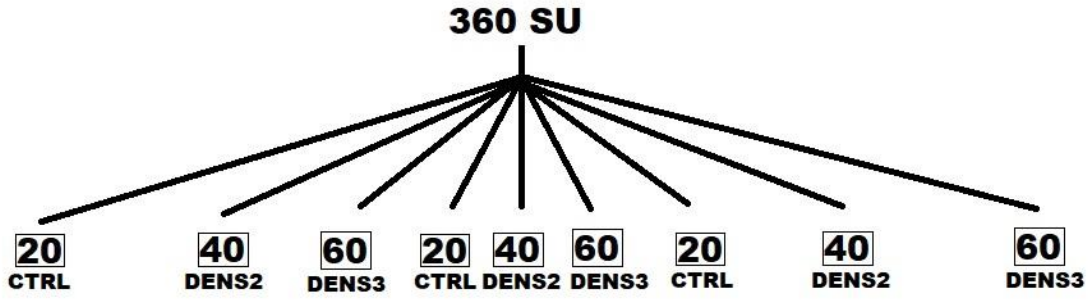

Fig. 2.6. Experimental design of laboratory conditions rearing density trial (SU = Sea urchin).

In this trial, the type of feed was equal to all treatments, and the amount of feed supplied was determined according to the biomass of each treatment, following a base of 2% $BW.d^{-1}$. Every 3 days, the tanks were cleaned, uneaten feed was determined and removed, and new feed was accurately weighed and replenished.

All sea urchins, from each treatment, were weighed individually at the beginning and at the end of the trial. An intermediate weighing of the totality of the sea urchins in each tank was carried out, one month after the beginning of the experiment, to follow the evolution of biomass.

This trial lasted 8 weeks.

2.5 Feeding behavior trial

Three wide angle video cameras (GoPro Hero 4), one per treatment, were placed in the top of the tanks, about 50 cm above the water surface, to allow an entire visualization of the tanks bottom, where the sea urchins were. Video cameras were configured to take one photography every 60 s. This feeding behavior trial started after the introduction of the feed, and recorded sea urchin interaction with the feed for 4 h.

2.6 Analytical methods

To determine the performance of sea urchins, the following formulas were used:

$$Wet\ Weight\ Gain\ (WWG\ \%) = \left[\frac{(t\ wet\ weight - (t-1)\ wet\ weight)}{(t-1)\ wet\ weight}\right] \times 100$$

$$Total\ Wet\ Weight\ Gain\ (TWWG\ \%) = \left[\frac{(t\ wet\ weight - t0\ wet\ weight)}{t0\ wet\ weight}\right] \times 100$$

$$Specific\ Growth\ Rate\ (SGR\ \%) = \left[\frac{(\ln t\ wet\ weight - \ln(t-1)\ weight)}{T\ (days)}\right] \times 100$$

$$Total\ Specific\ Growth\ Rate\ (TSGR\ \%) = \left[\frac{(\ln t\ wet\ weight - \ln(t0) wet\ weight)}{T\ (days\ between\ t\ and\ t0)}\right] \times 100$$

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Total\ feed\ intake\ (g)}{Total\ wet\ weight\ gain\ (g)}$$

$$Survival\ rate\ (\%) = \frac{Final\ number\ of\ surviving\ sea\ urchins}{Initial\ number\ of\ sea\ urchins} \times 100$$

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{Wet\ weight\ gain\ (g)}{Total\ protein\ intake\ (g)}$$

$$Gonadosomatic\ Index\ (GSI) = \frac{Gonads\ wet\ weight\ (g)}{Total\ wet\ weight\ (g)} \times 100$$

2.7 Histological analysis of *P. lividus* gonads

After fixation, the samples of *P. lividus* gonads were embedded in paraffin, from which 4 µm histological sections were made. Those samples were stained following the Hematoxylin Eosin (H&E) protocol and observed under an optical microscope.

The gonad maturity stage was determined following the method described by James and Siikavuopio (2012) and classified into one of 4 different maturity stages: Stage I: Post spawning; Stage II: Storage cells (nutritive phagocytes) growth; Stage III: Development of reproductive cells; Stage IV: Spawning and pre-spawning.

2.8 Statistical analysis

Before any statistical analysis, all the data obtained was tested for normality of distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test). All results obtained were statistically compared to determine the existence of potential differences between treatments (One-way ANOVA, when equal variances between treatments, or Welch's ANOVA, when unequal variances between treatments) with a 95% confidence interval, that is, $p < 0.05$. This statistical study was carried out with R® software and its "R Studio" package.

3. RESULTS

3.1. Farm-conditions nutritional trial

3.1.1. Ambiental parameters

During the farm-conditions trial, the sea urchins were exposed to natural photoperiod and water temperature. Thus, the photoperiod varied between 9.5L:14.5D and 11.25L:12.75D and the water temperature between 10.2ºC and 19.1ºC, with an average temperature of 16.1 ± 2.6 ºC and a salinity of 36-37‰.

3.1.2. Survival rate

The survival rate for sea urchins fed with Diet 1, Diet 2 and *Ulva* spp. was 99.33%, 100% and 100% respectively, and was not significantly different among the different treatment groups.

3.1.3. Somatic and gonadal growth

No statistically significant differences were found between treatments in the test diameter and wet weight of sea urchins during the different samplings carried out.

Table 3.1. *Paracentrotus lividus*, growth parameters at T=1, fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	T=1		
	D1	D2	CTRL
Test diameter (cm)	3.55 ± 0.34^{a}	3.45 ± 0.40^{a}	3.43 ± 0.37^{a}
Wet weight (g)	15.89 ± 3.42^{a}	14.92 ± 4.23^{a}	15.17 ± 3.72^{a}
Total wet weight gain (%)	16.44 ± 6.19^{ab}	22.55 ± 2.74^{b}	10.20 ± 4.75^{a}
Specific growth rate (%)	0.49 ± 0.17^{ab}	0.66 ± 0.07^{b}	0.31 ± 0.14^{a}
Total Specific growth rate (%)	0.49 ± 0.17^{ab}	0.66 ± 0.07^{b}	0.31 ± 0.14^{a}

n=30. (mean ± SD). Letters a, b represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

At T=1 and T=2 (Table 3.1 and Table 3.3), the sea urchins from the treatment D2 showed a significantly higher total wet weight gain than the individuals from the control treatment (T=1, p=0.044; T=2, p=0.008), however, no statistically significant difference was found between the total wet weight gain of the sea urchins from treatment D1 and D2 (T=1, p=0.329; T=2, p=0.264), nor of the sea urchins from treatment D1 and control treatment (T=1, p=0.316; T=2, p=0.062). At T=3 (Table 3.4), the total wet weight gain of the sea urchins from the treatment D2 continued to be significantly higher than that of the control treatment individuals (p<0.001), however, it also became significantly higher than that of the sea urchins of treatment D1 (p=0.005). Likewise, the total wet weight gain of the sea urchins from the treatment D1 was significantly higher than that of the control individuals (p=0.005). At T=4 (Table 3.5), the total wet weight gain of the sea urchins from treatment D1 did not show statistically significant differences from the total wet weight gain of the sea urchins from treatment D2 (p=0.186), however, both showed a significantly total wet weight gain higher than that of the control treatment individuals (D1, p=0.029; D2, p=0.003) (Figure 3.1).

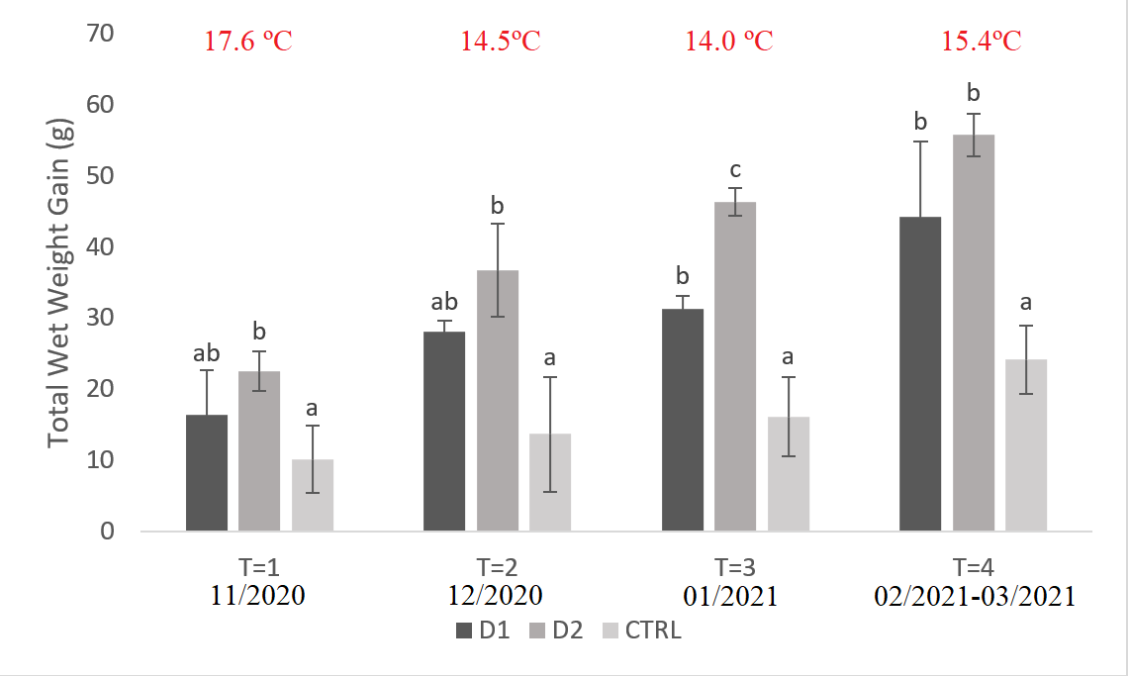

Fig. 3.1. *Paracentrotus lividus* TWWG from T=0, fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.). Letters a, b, c represent a significant difference (p<0.05) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences (p>0.05).

In the first 3 samplings, after the introduction of the different diets, the gonadosomatic index of the sea urchins from the treatment D1 did not show any statistically significant difference with the sea urchins from the treatment D2 (T=1, p=0.687; T= 2, p=0.550; T=3, p=0.922) (Table 3.2). In contrast, in the final sample, T=4, the gonadosomatic index of the sea urchins from treatment D1 was significantly higher than that of individuals from treatment D2 (p=0.021) (Figure 3.2). Once the different diets were introduced, and this throughout the experiment, significantly higher gonadosomatic indices were observed in sea urchins fed with formulated diets (treatments D1 and D2), when compared to the gonadosomatic index of sea urchins from the control treatment, fed with *Ulva* spp. at T=1 (D1, p=0.007; D2, p=0.019), T=2 (D1, p<0.001; D2, p<0.001), T=3 (D1, p<0.001; D2, p=0.002) and T=4 (D1, p<0.001; D2, p<0.001).

Table 3.2. *Paracentrotus lividus* GSI fed with different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	GSI		
	D1	D2	CTRL
T=1	0.12 ± 0.02^{b}	0.11 ± 0.02^{b}	0.05 ± 0.01^{a}
T=2	0.14 ± 0.00^{b}	0.15 ± 0.02^{b}	0.04 ± 0.01^{a}
T=3	0.18 ± 0.03^{b}	0.14 ± 0.03^{b}	0.04 ± 0.00^{a}
T=4	0.20 ± 0.01^{c}	0.17 ± 0.01^{b}	0.04 ± 0.01^{a}

n=3 (mean ± SD). Letters a, b, c represent a significant difference (p<0.05) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences (p>0.05).

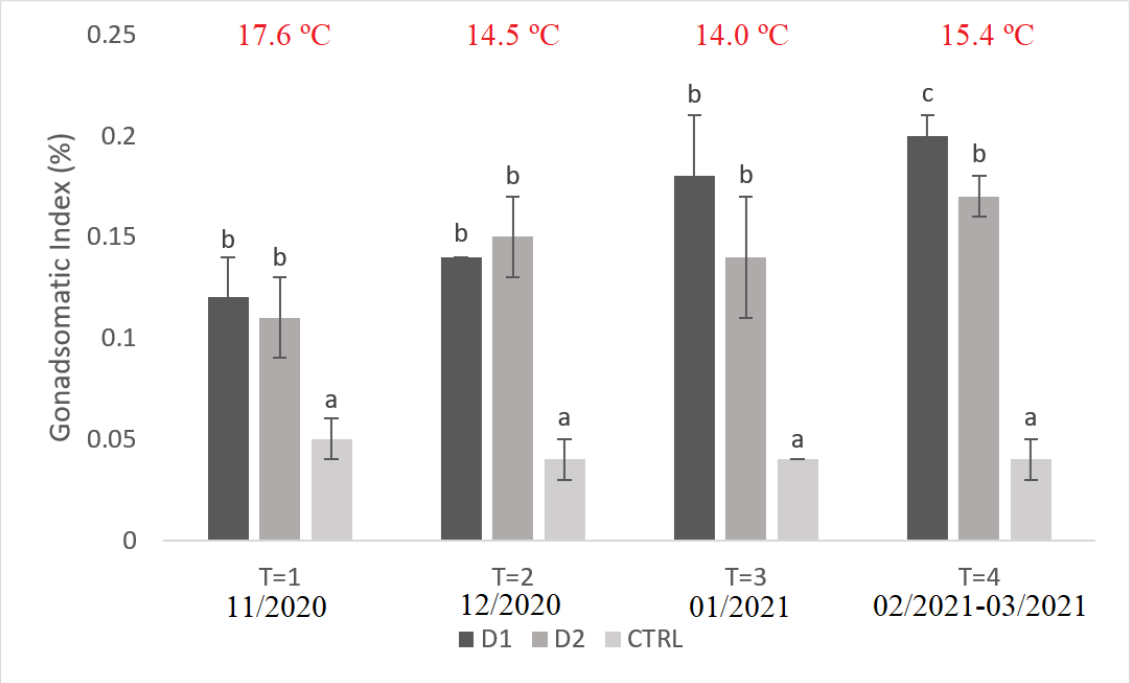

Fig. 3.2. *Paracentrotus lividus* GSI along the trial, fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.). Letters a, b, c represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

As for the total wet weight gain, the specific growth rate of the sea urchins from the treatment D2 was significantly higher than the specific growth rate of the sea urchins from the control treatment, at T=1 (Table 3.1) ($p=0.047$) but similar with the specific growth rate of the individuals in treatment D1 ($p=0.350$). Likewise, the specific growth rate of the sea urchins from the treatment D1 was not significantly different from that of the control treatment individuals ($p=0.317$). In the rest of the experiment, there were no statistically significant differences between the specific growth rates of the sea urchins from the different treatments.

Table 3.3. *Paracentrotus lividus,* growth parameters at T=2 fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	T=2		
	D1	D2	CTRL
Test diameter (cm)	3.74 ± 0.33^{a}	3.65 ± 0.36^{a}	3.61 ± 0.33^{a}
Wet weight (g)	17.49 ± 3.37^{a}	16.68 ± 4.35^{a}	15.62 ± 3.40^{a}
Total wet weight gain (%)	28.07 ± 1.51^{ab}	36.74 ± 6.58^{b}	13.70 ± 8.08^{a}
Specific growth rate (%)	0.25 ± 0.11^{a}	0.29 ± 0.13^{a}	0.08 ± 0.09^{a}
Total Specific growth rate (%)	0.36 ± 0.02^{ab}	0.45 ± 0.07^{b}	0.18 ± 0.10^{a}

n=30. (mean ± SD). Letters a, b represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

Between T=0 and T=2 (Table 3.3), the total specific growth rate of the sea urchins in the treatment D2 was significantly higher than that of the individuals in the control treatment ($p=0.009$), without, however, showing statistically significant differences with the total specific growth rate of sea urchins from treatment D1 ($p=0.325$). Likewise, there was no statistically significant difference between the total specific growth rate of sea urchins from the treatment D1 and the control treatment individuals ($p=0.060$). Between T=0 and T=3 (Table 3.4), the total specific growth rate of sea urchins from treatment D2 remained significantly higher than that of individuals from the control treatment ($p<0.001$), however, it also became significantly higher that of the individuals in treatment D1 ($p=0.009$). The total specific growth rate of the sea urchins from the treatment D1 was also significantly higher than that of the control treatment individuals ($p=0.005$). Between T=0 and T=4 (Table 3.5), the total specific growth rate of the sea urchins from the treatment D2 no longer showed a statistically significant difference with that of the sea urchins from the treatment D1, however, both remained significantly higher to the total specific growth rate of the individuals in the control treatment (D1, $p=0.024$; D2, $p=0.003$) (Figure 3.3).

Table 3.4. *Paracentrotus lividus*, growth parameters at T=3 fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	T=3		
	D1	D2	CTRL
Test diameter (cm)	3.74 ± 0.34[a]	3.71 ± 0.34[a]	3.61 ± 0.32[a]
Wet weight (g)	17.92 ± 3.29[a]	17.84 ± 4.18[a]	15.96 ± 3.51[a]
Total wet weight gain (%)	31.25 ± 1.89[b]	46.32 ± 1.94[c]	16.11 ± 5.57[a]
Specific growth rate (%)	0.08 ± 0.06[a]	0.23 ± 0.17[a]	0.07 ± 0.18[a]
Total Specific growth rate (%)	0.27 ± 0.01[b]	0.38 ± 0.01[c]	0.15 ± 0.05[a]

n=30. (mean ± SD). Letters a, b, c represent a significant difference ($p<0.05$) In the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

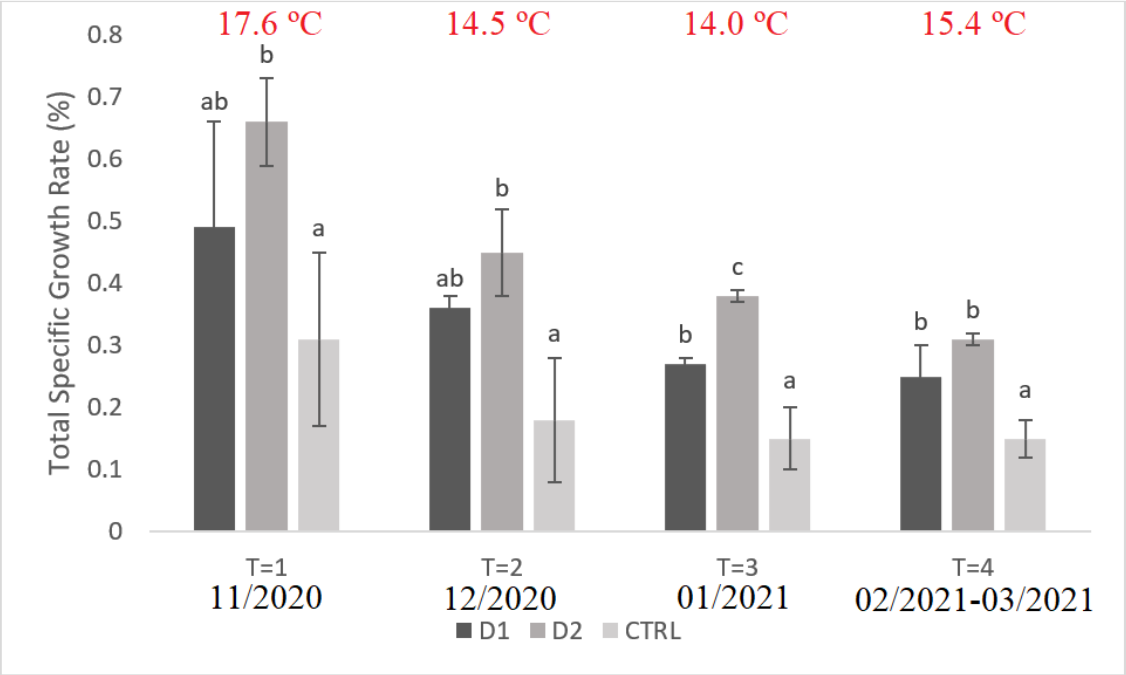

Fig. 3.3. *Paracentrotus lividus* TSGR from T=0, fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.). Letters a, b, c represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

Table 3.5. *Paracentrotus lividus,* growth parameters at T=4 fed with three different diets, under farm-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	T=4		
	D1	D2	CTRL
Test diameter (cm)	3.94 ± 0.37^{a}	3.90 ± 0.36^{a}	3.74 ± 0.33^{a}
Wet weight (g)	19.71 ± 3.70^{a}	19.00 ± 4.64^{a}	17.08 ± 3.71^{a}
Total wet weight gain (%)	44.19 ± 10.64^{b}	55.73 ± 2.98^{b}	24.19 ± 4.82^{a}
Specific growth rate (%)	0.21 ± 0.14^{a}	0.14 ± 0.02^{a}	0.15 ± 0.07^{a}
Total Specific growth rate (%)	0.25 ± 0.05^{b}	0.31 ± 0.01^{b}	0.15 ± 0.03^{a}

n=30. (mean ± SD). Letters a, b represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

3.1.4. Histological analysis

Histological analysis of the sea urchin gonads did not show any difference between the different treatments. At the end of the experiment, all individuals used in the histological analysis were in phase IV: Spawning and pre-spawning (Figure 3.4).

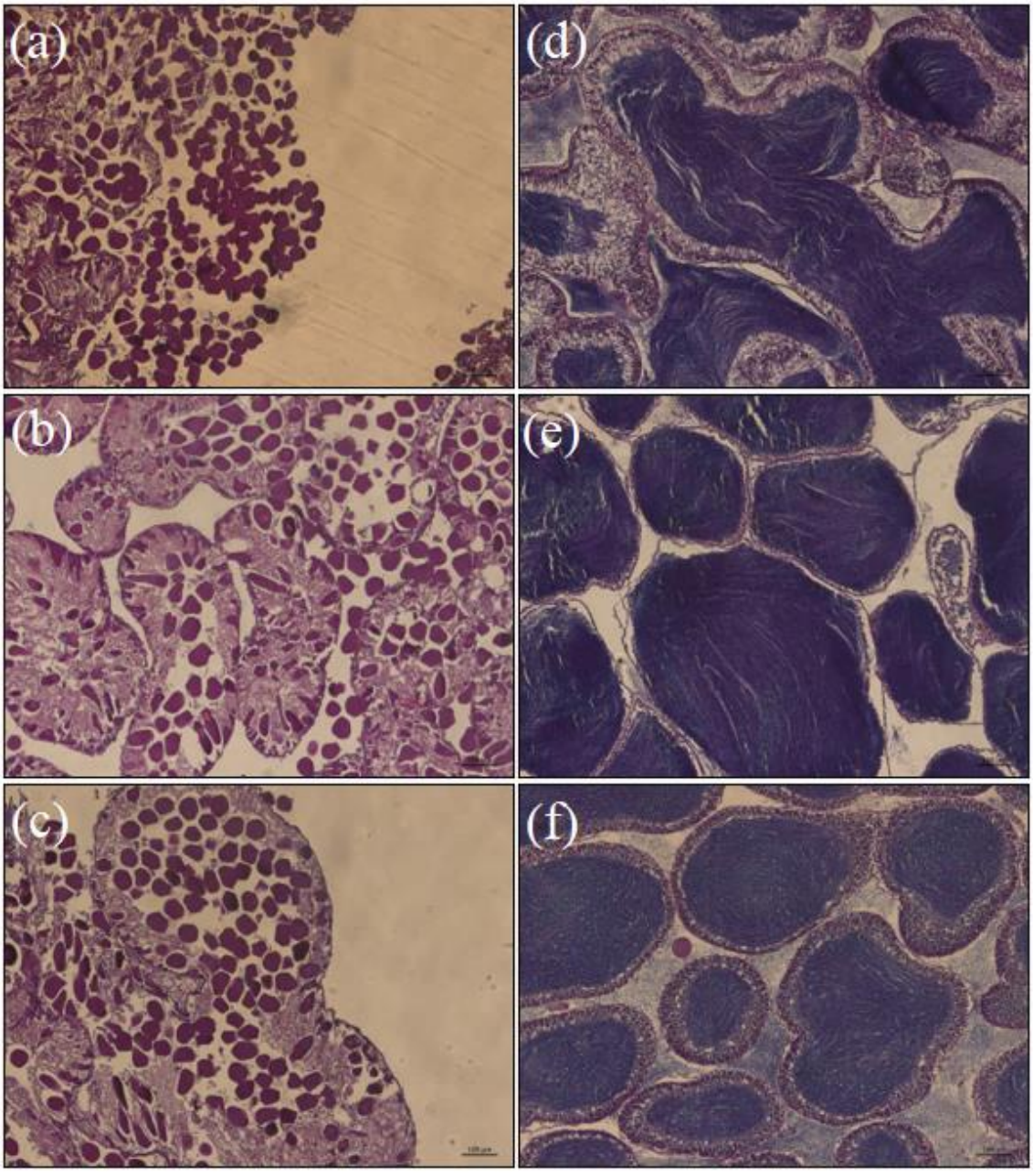

Fig. 3.4. Histological section of gonad from *Paracentrotus lividus* at the end of the trial, under farm-conditions. Images a, b and c represent gonads from females fed with Diet 1, Diet 2 and *Ulva* spp. respectively. Images d, e and f represent gonads from males fed with Diet 1, Diet 2 and *Ulva* spp. respectively (total magnification: 100x).

3.2. Laboratory-conditions feeding trial

3.2.1. Ambiental parameters

During this lab-conditions test, the photoperiod was 9L:15D, and the mean water temperature was 16.6 ± 1.88ºC. The average concentration of dissolved oxygen in the water was 8.14 ± 1.03 mg l^{-1}, for an average oxygen saturation percentage of 89.73 ± 7.70%.

3.2.2. Survival rate

The survival rate for sea urchins fed with Diet 1, Diet 2 and *Ulva* spp. was 100%, 98.33% and 100% respectively, and was not significantly different among the different treatment groups.

3.2.3. Somatic growth

At the end of the experiment, the wet weight gain of the sea urchins from treatment D2 was significantly higher than that of the individuals from control treatment (p=0.014) (Figure 3.5). However, no significant differences were found between the wet weight gain of the sea urchins from the treatments D2 and D1 (p=0.251), nor between the wet weight gain of the sea urchins from the treatment D1 and the control treatment (p=0.122) (Table 3.6).

Similarly, the specific growth rate of the individuals in the treatment D2 was significantly higher than that of the sea urchins in the control treatment (p=0.014) but showed no statistically significant differences with the specific growth rate of the individuals in the treatment D1 (p=0.271) (Figure 3.5). Furthermore, the statistical study between the specific growth rate of the sea urchins from the D1 treatment and the specific growth rate of the sea urchins from the control treatment did not show any significant difference (p=0.108) (Table 3.6).

Table 3.6. WWG (%) and SGR (%) of the sea urchins from the three treatment groups, under laboratory-conditions (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	D1	**D2**	**CTRL**
Wet weight gain (%)	19.89 ± 1.28^{ab}	25.81 ± 4.83^{b}	12.08 ± 2.81^{a}
Specific growth rate (%)	0.22 ± 0.01^{ab}	0.28 ± 0.05^{b}	0.14 ± 0.03^{a}

n=20. (mean ± SD). Letters a, b represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

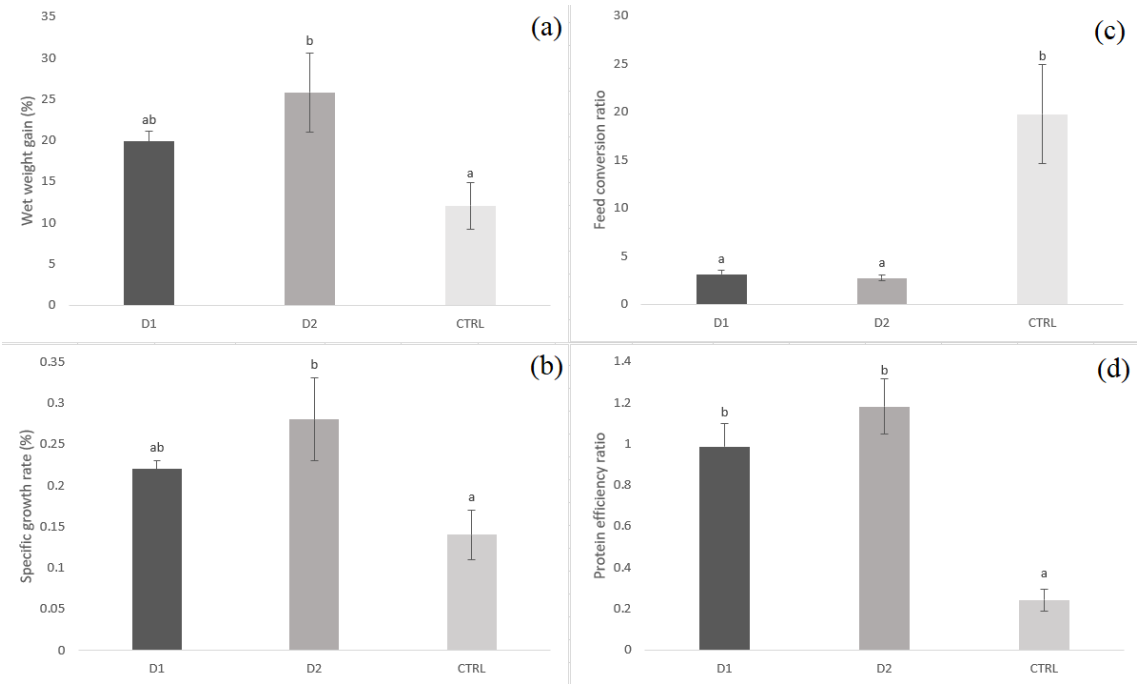

Fig. 3.5. Growth and nutritional performances of *Paracentrotus lividus* under laboratory-conditions, fed with three different diets: a) WWG (%), b) SGR (%), c) FCR, d) PER. (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.). Letters a, b represent a significant difference ($p<0.05$) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences ($p>0.05$).

3.2.4. Nutritional performances

The feed conversion ratio of sea urchins fed with formulated diets did not show any significant difference (p=0.990), although, significant differences were founded with feed conversion ratio of sea urchins fed with *Ulva* spp. (Figure 3.5) In fact, the feed conversion rate of sea urchins fed with *Ulva* spp. was significantly higher than that of individuals from treatment D1 (p=0.004) and individuals from treatment D2 (p=0.003) (Table 3.7).

The protein efficiency ratio of sea urchins fed with formulated diets did not show any significant difference (p=0.235) (Figure 3.5). Conversely, the supposed protein efficiency ratio of sea urchins fed with *Ulva* spp. was significantly lower than that of individuals from treatment D1 (p<0.001) and individuals from treatment D2 (p<0.001) (Table 3.7).

Table 3.7. Feed conversion ratio and protein efficiency ratio of the sea urchins from the three treatment groups (D1: Diet 1, D2: Diet 2, CTRL: *Ulva* spp.).

	D1	**D2**	**CTRL**
Feed conversion ratio	3.15 ± 0.39^{a}	2.75 ± 0.34^{a}	19.77 ± 5.18^{b}
Protein efficiency ratio	0.99 ± 0.11^{b}	1.18 ± 0.13^{b}	$0.24 \pm .0.05^{a}$*

(mean ± SD). Letters a, b represent a significant difference (p<0.05) in the means of sea urchins fed the different diets. Same letter in the line stand for no significative differences (p>0.05). The asterisk (*) represents an estimated value.

3.3. Laboratory-conditions density trial

3.3.1. Ambiental parameters

During this test under lab-conditions, the photoperiod was 9L:15D, and the mean water temperature was 16.2 ± 1.29ºC. The average concentration of dissolved oxygen in the water was 7.6 ± 0.72 mg l^{-1}, for an average oxygen saturation percentage of 94.1 ± 7.63%.

3.3.2. Survival rate

The mean survival rate for sea urchins reared at 89 ind.m^{-2}, 179 ind.m^{-2}, and 268 ind.m^{-2} was 100%; no mortality was registered during the experiment.

3.3.3. Somatic growth

No statistically significant differences were found in wet weight gain nor specific growth ratio on sea urchins from the different density treatments. The average wet weight gain at the end of this experiment was 22.75 ± 2.75 %, and the average specific growth ratio was 0.32 ± 0.035 (Table 3.8).

Table 3.8. Wet weight gain (%) and specific growth rate (%) of the sea urchins from the three treatment groups, under laboratory-conditions (Dens1: Density 1=89 ind.m^{-2}, Dens2: Density 2=179 ind.m^{-2}, Dens3: Density 3=268 ind.m^{-2}).

	Dens1	**Dens2**	**Dens3**
Wet weight gain (%)	24.42 ± 0.70^{a}	21.34 ± 2.30^{a}	22.47 ± 3.47^{a}
Specific growth rate (%)	0.34 ± 0.01^{a}	0.30 ± 0.03^{a}	0.32 ± 0.04^{a}

n=20, 40 & 60 respectively. (mean ± SD). Same letter in the line stand for no significative differences (p>0.05).

3.3.4. Nutritional performances

Both the feed conversion ratio and the protein efficiency ratio of sea urchins showed no statistically significant differences between the different density treatments. The average feed conversion ratio was 3.97 ± 2.28 and the average protein efficiency ratio was 0.97 ± 0.13 (mean ± SD) (Table 3.9).

Table 3.9. Feed conversion ratio and protein efficiency ratio of the sea urchins from the three treatment groups, under laboratory-conditions (Dens1: Density 1=89 ind.m^{-2}, Dens2: Density 2=179 ind.m^{-2}, Dens3: Density 3=268 ind.m^{-2}).

	Dens1	**Dens2**	**Dens3**
Feed conversion ratio	2.07 ± 0.97^{a}	4.02 ± 1.30^{a}	5.82 ± 0.04^{a}
Protein efficiency ratio	1.01 ± 0.10^{a}	0.91 ± 0.15^{a}	0.98 ± 0.13^{a}

(mean ± SD). Same letter in the line stand for no significative differences (p>0.05).

4. DISCUSSION

4.1 Survival rate

The survival rate recorded at the end of the experiment was high, both under laboratory conditions (99.4 ± 1.6 %) and under farm conditions (99.8 ± 0.6 %). This result is similar to that obtained by Cirino *et al*. (2017) and Heflin *et al*. (2016) with *Paracentrotus lividus* and *Lytechinus variegatus* respectively, under laboratory conditions, where the survival rate of both experiments was 100%. As the survival rate was high and was not significantly impacted between treatments, we can state that the different diets were not harmful for sea urchins' health, and the mortality was due to the handling during samplings and the cleaning of the tanks. These results are in line with those found by Zupo *et al*. (2018), which tested the effect of fresh and formulated diets on *P. lividus*, but also correspond to the results obtained in nutritional trials in other species of sea urchins, such as *Tripneustes gratilla* (Cyrus *et al*., 2012; Onomu *et al.,* 2020), in *L. variegatus* (Heflin *et al*., 2016) and in *Evechinus chloroticus* (Barker *et al*., 1998).

4.2 Feeding behavior, culture system and rearing density

The sea urchin is an animal that moves and feeds slowly (Spirlet *et al.,* 2001), so its feeding movements is not very marked. During the 4 h in which the feeding behavior of sea urchins was filmed, there was little interaction between the individuals and the different feeds, whether fresh *Ulva* spp. or inert diets. The sea urchins preferred to stay in the corners and sides of the baskets, rather than go towards the feed, probably being part of the defensive strategy of these animals, as previously described by Tegner & Dayton (1977). It should be noted that the feeding behavior was observed during the 4 hours after the introduction of the different feeds, and that such behavior may eventually vary as a function of the time during which the feed was submerged. Since not all sea urchins detach themselves towards the feed, feed was started to be placed directly on top of them, which resulted in higher ingestion rates, and consequently, less wasted feed that could degrade water quality (Spirlet *et al.,* 2001). The inert diets were well accepted by the sea urchins, probably due to the inclusion of *Ulva* spp. in their formulation. Cyrus *et al*. (2015) found that the inclusion of 20% of *Ulva* spp. in formulated diets (the same amount of *Ulva* spp. that was added in the inert diets tested in this work) improved the palatability of the feed, but also the feed consumption rate and the daily digestible protein intake.

Their work proved that *Ulva* spp. increased the acceptability of the formulated diets by the sea urchins, thus acting as a feeding stimulant.

The new tested cultivation system, initially developed for oyster rearing, proved to be effective in the cultivation of sea urchins under farm conditions. In fact, due to the sea urchins being able to attach themselves to all the walls of the basket, they had more surface to evolve than in the systems usually used for their rearing. The size of the mesh in the basket was ideal as it was small enough for the sea urchins to be able to attach themselves in and stop the feed from falling out of the basket, but big enough to let the feces going through. The presence of two openings, one at each end of the basket, facilitated the feeding and maintenance process, and as they were designed to withstand high energy environments, these baskets seem to be suitable for the rearing of sea urchins in land-based aquacultures, but also in open water aquaculture.

In the nutritional assay carried out under laboratory conditions, no significant differences were observed between the groups of the different densities tested, both in survival rate, somatic growth, and in nutritional performance. The results obtained show that, for the three densities tested (89, 179 and 268 ind.m^{-2}), the rearing density does not impact the health, growth, and nutritional performance of the sea urchins, and that such densities can be applied in an echinoculture. However, it is important to note that very high rearing densities of sea urchins can have a negative impact on their growth. Juiniu-Meñez *et al*. (2008) found that sea urchins reared at higher densities (750 ind.m^{-2}) had a lower survival rate and a lower gonadosomatic index than sea urchins cultivated at lower densities (250 ind.m^{-2}), probably due to unequal access to the feed, or a deterioration of water quality in high-density rearing tanks.

4.3 The effect of feed in sea urchin somatic growth

In this study, sea urchins fed fresh *Ulva* spp. had somatic characteristics (wet weight and test diameter) comparable to sea urchins fed inert diets supplemented with *Ulva* spp. (20% *Ulva* spp.). These results are in agreement with the results obtained by Shpigel *et al.* (2005) in a nutritional trial on *P. lividus*, but it has also been observed in other sea urchin species. In fact, Asia *et al.* (2012) found that the somatic characteristics of juveniles *T. gratilla*, fed fresh *Sargassum*, were similar to individuals fed inert *Sargassum*-supplemented diets. Likewise, such results were observed in juveniles *Strongylocentrotus dorebachiensis* (Daggett *et al.*, 2005) and *Evechinus chloroticus* (Barker *et al*., 1998),

both fed with fresh macroalgae or inert diets. The absence of somatic differences between sea urchins fed fresh and formulated diets may be due to the energy being allocated essentially to gonadosomatic growth (Harris & Eddy, 2015).

In both trials, under laboratory and farm conditions, inert Diet 2 showed better results in sea urchins wet weight gain and specific growth rate than fresh *Ulva spp*. This result may be directly related to the difference in protein content between this formulated diet and fresh *Ulva* spp., as protein is fundamental for somatic growth (Schlosser *et al*., 2005; Cyrus *et al*., 2015). In fact, the amount of protein in fresh *Ulva* spp. previously found by Ferreira *et al.* (2021) was 22.1% DM, while the inert Diet 2 tested in the present work had 31.2% DM. Cyrus *et al*. (2012) obtained similar results with *T. gratilla,* where the animals fed formulated diets supplemented with *Ulva* spp., richer in protein than fresh *Ulva* spp., showed enhanced growth and development. In that same trial, the energy relative to the different diets could also be responsible for such differences, and this could also be the case in this trial as the energy of fresh *Ulva* spp. found by Ferreira *et al.* (2021) (11.03 kJ/g) was significantly lower than the energy of the inert Diets 1 and 2 (15.75 and 15.93 kJ/g, respectively). Heflin *et al*. (2016) developed a predictive model to the total wet weight gain (TWWG) in *Lytechinus variegatus*, as a function of the protein content of the feed provided and concluded that the sea urchins TWWG increased with the increase in protein content (between 10 and 40% DM) present in the diet. The works carried out by Hammer *et al* (2004; 2012) revealed that the minimum percentage of protein recommended for good growth and survival of juveniles *L. variegatus* was 21% DM, and that the best results in growth and nutritional performance of this same species was on diets with 31% DM of protein.

In the test under laboratory conditions, there were no statistically significant differences in wet weight gain and specific growth rate between sea urchins fed inert Diet 1 and fresh *Ulva* spp.. However, in the trial under farm conditions, the growth differences between these two treatments were observed from T=3, where sea urchins fed with inert Diet 1 showed greater TWWG and higher total specific growth rate. The absence of significant growth differences between these two treatments in the test under laboratory conditions may be related to its duration. In fact, this trial lasted approximately 10 weeks, whereas significant differences between these two treatments only appeared after 14 weeks in the trial under farm conditions. As sea urchins fed with inert Diet 2 showed a statistically greater wet weight gain and specific growth rate than sea urchins fed with fresh *Ulva* spp.

after 10 weeks, we can, however, suppose that inert Diet 2 has a faster positive effect on somatic growth in *P. lividus* than inert Diet 1.

The specific growth rate (SGR) of sea urchins in the farm-conditions trial showed a decrease in the middle of the experiment and until the end, common to all treatments. This decrease in the SGR is related to the decrease in water temperature, typical of Winter months. While the average water temperature in the first month of the trial was 17.6 ± 1.3 ºC, the average water temperature in the last three months was 14.7 ± 1.6 ºC. The relationship between temperature and somatic growth of sea urchins has been previously studied by several authors, who found that lower temperatures slow down metabolism and respiration (Spirlet *et al*., 2000; Brockington & Clarke, 2001; McBride *et al*., 1997), and are responsible for reduced growth rates (Siijavuopio *et al*., 2006), as in the trial reported by Spirlet *et al.* (2000), where, at 12ºC, no somatic growth was registered in *P. lividus*.

The total specific growth rate of sea urchins fed inert Diet 2 also decreased due to temperature, however this remained significantly higher than the total specific growth rate of sea urchins fed fresh *Ulva spp*. This result means that the inert Diet 2 can guarantee somatic growth even at low temperatures, which makes it more suitable for outdoor, under farm conditions, echinoculture.

4.4 The effect of feed in sea urchin gonads

Gonadal growth is due to the accumulation of significant amounts of nutrients by the nutritive phagocytes (NP) in the gonads (Kalachev & Yurchenko, 2016), and is directly influenced by the quantity and quality of feed available (Cirino *et al*., 2017). In this trial, under farm conditions, the effect of inert diets was constantly verified throughout the experiment, in all the different samples: sea urchins fed the inert diets had significantly higher gonadosomatic indices (GSI) than sea urchins fed with fresh *Ulva spp*. These results correspond to those obtained by several authors in *P. lividus* (Grosjean & Jangoux, 2001; Vizzini *et al.,* 2018; Prato *et al.,* 2018; Shpigel *et al.,* 2005; Spirlet *et al.,* 2001; McBride *et al*., 2004; Schlosser *et al*., 2005) but also in other species of sea urchins, such as *T. gratilla* (Onomu *et al*., 2020; Cyrus *et al.,* 2012), and *L. variegatus* (Hammer *et al*., 2004). As for somatic growth, gonadal growth depends mainly on factors such as the amount of energy and protein in the diet (Schlosser *et al*., 2005). As previously mentioned, the energy value of the inert diets tested in this trial was higher than the

energetic value of fresh *Ulva* spp. found by Ferreira *et al.* (2021), and the same could be found for protein content, as the percentage of protein present in the inert diets was almost 50% higher than in fresh *Ulva spp*. Based on the literature, it is possible to suppose that the higher amount of protein and energy in the inert diets was responsible for an increase in the gonadosomatic index of the sea urchins (Heflin *et al*., 2016). In contrast to somatic growth, the gonadal growth of sea urchins fed the inert diets was not negatively impacted by the decrease in temperature throughout the experiment, which again shows that both inert diets are adequate for *P. lividus* rearing, under farm conditions, where the temperature is likely to vary throughout the year.

In the last sampling, the sea urchins fed the inert Diet 1 showed higher gonadosomatic indices than the sea urchins fed the other diets. In the other hand, the results previously obtained in this work showed that sea urchins fed inert Diet 2 had significantly higher somatic growth than sea urchins fed fresh *Ulva* spp., which was only the case of sea urchins fed with inert Diet 1, in the last two samplings. As energy is allocated to somatic growth and gonadal growth, we can assume that sea urchins fed inert Diet 1 allocated more energy to gonadal growth than somatic growth, while sea urchins fed inert Diet 2 allocated more energy in somatic growth than gonadal growth. Previous studies have associated the difference in energy allocation with the age and size of sea urchins: younger (< 20 mm) size class individuals allocated more energy to somatic growth than to gonadal growth (Fernandez & Boudoresque, 2000) and the opposite occurred in older, upper-size class individuals (Shpigel *et al*., 2005; McCarron *et al*., 2009). However, in the present work there were no statistically significant differences in the size of the sea urchins, that is, they all belonged to the same size class.

Histological analysis of *P. lividus* gonads at the end of the trial under farm conditions did not show any statistically significant difference between the three treatments. Sea urchins fed the inert diets and fresh *Ulva* spp. were all in phase IV: Spawning and pre-spawning. This result is in line with that obtained by Cyrus *et al*. (2012) and Shpigel *et al*. (2018), who performed the histological analysis of *T. gratilla* gonads, fed with fresh *Ulva* spp. and inert diets including *Ulva spp*. In the other hand, Onomu *et al*. (2020) found that inert diets with 20% of *Ulva* spp. could increase the rate of gonad maturation of *T. gratilla*. It could probably be justified by the differences in the protein content of the diets, as the formulated diet used by Onomu *et al*. (2020) had a lower protein content (25.7% DM) than the formulated diets used in the present work (31-33% DM). The absence of

differences in gonad maturity status between sea urchins fed fresh *Ulva* spp. and sea urchins fed the two inert diets suggests that these inert diets do not alter the rate of gonad maturation of *P. lividus*. This is particularly important as the market acceptability differs with the reproductive stage of a sea urchin (Unuma, 2002).

4.5 The effect of feed in sea urchin nutritional performances

The nutritional performances observed in the trial under laboratory conditions showed significant differences between sea urchins fed the inert diets and sea urchins fed fresh *Ulva spp*. In fact, the feed conversion ratio (FCR) of sea urchins fed fresh *Ulva* spp. was 6 to 7 times higher than that of sea urchins fed inert diets. This is mainly justified by the high moisture content of fresh *Ulva* spp., which forces sea urchins to ingest larger volumes of feed, and to devote more energy to handling and chewing feed (Spirlet *et al*., 2001).

In fact, each sea urchin fed one of the two inert diets ate, on average, approximately 0.13 g of feed per day, while each sea urchin fed fresh *Ulva* spp. ate, on average, approximately 0.43 g of feed per day. As inert diets have about 93-94% dry matter and fresh *Ulva* spp. only 12.8%, this corresponds to approximately 0.12 g (dry weight) of feed ingested per day for each sea urchin fed one of the two inert diets, against about 0.06 g (dry weight) of feed ingested per day for each sea urchin fed fresh *Ulva spp*. These results are similar to those obtained by Cyrus *et al*. (2012) who then concluded that high protein content, together with higher feed intake (dry weight) contributed to greater somatic growth in sea urchins fed the inert diets.

Protein is the most expensive nutrient in formulated diets (Fleming *et al*., 1996), so it is very important to maximize the protein efficiency ratio. In this trial, under laboratory conditions, the protein efficiency ratio (PER) of sea urchins fed the inert diets were significantly higher than the PER of sea urchins fed fresh *Ulva* spp., with values 3 to 4 times higher than those of the PER of sea urchins fed with fresh *Ulva* spp., which was, on average, 0.24 (estimated PER, using the protein content found by Ferreira *et al.* 2021). The PER of sea urchins fed with inert diets were within the values announced by the predictive model developed by Heflin *et al*. (2016) for *L. variegatus*. In the work performed by Schlosser *et al*. (2005), the PER was also higher in *P. lividus* fed a formulated diet with 15% Kelp than in sea urchins fed fresh *Ulva spp*.

The results of nutritional performance obtained in the present study support the idea that inert diets are nutritionally richer, protein is used more efficiently and ensure greater somatic and gonadal development, with smaller amounts of feed supplied than with fresh *Ulva* spp..

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Both two inert diets tested in this work, supplemented with 20% *Ulva* spp., were well accepted by sea urchins, and had no impact on their health. These two inert diets had a positive effect on the somatic and gonadal growth of juveniles *P. lividus*, when compared to juveniles *P. lividus* fed on fresh *Ulva spp*. The inert Diet 1 was significantly more effective in the gonadal growth of sea urchins, while the inert Diet 2 was more effective in the somatic growth. Similarly, juveniles *P. lividus* fed the inert diets had lower FCR and higher PER than sea urchins fed fresh *Ulva spp*. Furthermore, unlike fresh *Ulva* spp., the inert diets tested in this trial proved effective even in the months when the water temperature was lower. The baskets initially intended for the cultivation of oysters also proved to be suitable for the cultivation of sea urchins as they ensured good fixation to the sea urchins, good circulation of water and organic matter, and facilitated their maintenance. As the cultivation of sea urchins is intended for human consumption, it would be interesting to evaluate the amino acid composition in the gonads of *P. lividus* fed these inert diets in future studies, as amino acids influence the taste of the gonads, which is a of the main determining factors in its commercialization, such as texture and color. In parallel, other studies could evaluate the reproductive capacity and quality of gametes of *P. lividus* fed these inert diets, in order to determine if these diets are suitable for the reproduction of sea urchins to obtain larvae and quantify the loss of protein of the inert diets in the water. The results obtained in this trial demonstrated that inert diets supplemented with 20% of *Ulva* spp. are suitable for feeding juveniles *P. lividus*, ensuring good health and good somatic and gonadal development, and can also be used in echinoculture under farm conditions, as they are effective even with natural variations in water temperature throughout the year.

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7. APPENDICES

Appendix I – Plastic bags with 500 g of *Ulva* spp. before freezing (control treatment).

Appendix II – Exemple of sea urchins biometric sampling. The sea urchins were weighed one by one, then the test diameter was determined using “ImageJ” software.

Appendix III – *Paracentrotus lividus* gonads (female) at the end of the farm-conditions trial, fed with an inert diet.